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(54) **Title:**
MODULATION OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) EXPRESSION

(57) **Abstract:**
Disclosed herein are antisense compounds and methods for decreasing STAT3 mRNA and protein expression. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate hyperproliferative diseases.



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MODULATION OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) EXPRESSION

Sequence Listing

5 The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0142WOSEQ.txt created March 29, 2012 which is 672 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

10 Field

In certain embodiments provided are methods, compounds, and compositions for inhibiting expression of STAT3 mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate hyperproliferative diseases.

15 Background

The STAT (signal transducers and activators of transcription) family of proteins are DNA-binding proteins that play a dual role in signal transduction and activation of transcription. Presently, there are six distinct members of the STAT family (STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6) and several isoforms (STAT1 α , STAT1 β , STAT3 α and STAT3 β). The activities of the STATs are modulated by various
20 cytokines and mitogenic stimuli. Binding of a cytokine to its receptor results in the activation of Janus protein tyrosine kinases (JAKs) associated with these receptors. This phosphorylates STAT, resulting in translocation to the nucleus and transcriptional activation of STAT responsive genes. Phosphorylation on a specific tyrosine residue on the STATs results in their activation, resulting in the formation of homodimers and/or heterodimers of STAT which bind to specific gene promoter sequences. Events mediated by
25 cytokines through STAT activation include cell proliferation and differentiation and prevention of apoptosis.

The specificity of STAT activation is due to specific cytokines, i.e., each STAT is responsive to a small number of specific cytokines. Other non-cytokine signaling molecules, such as growth factors, have also been found to activate STATs. Binding of these factors to a cell surface receptor associated with protein tyrosine kinase also results in phosphorylation of STAT.

30 STAT3 (also acute phase response factor (APRF)), in particular, has been found to be responsive to interleukin-6 (IL-6) as well as epidermal growth factor (EGF) (Darnell, Jr., J.E., et al., Science, 1994, 264, 1415-1421). In addition, STAT3 has been found to have an important role in signal transduction by interferons (Yang, C.-H., et al., Proc. Natl. Acad. Sci. USA, 1998, 95, 5568-5572). Evidence exists suggesting that STAT3 may be regulated by the MAPK pathway. ERK2 induces serine phosphorylation and
35 also associates with STAT3 (Jain, N., et al., Oncogene, 1998, 17, 3157-3167).

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STAT3 is expressed in most cell types (Zhong, Z., et al., Proc. Natl. Acad. Sci. USA, 1994, 91, 4806-4810). It induces the expression of genes involved in response to tissue injury and inflammation. STAT3 has also been shown to prevent apoptosis through the expression of bcl-2 (Fukada, T., et al., Immunity, 1996, 5, 449-460).

5 Recently, STAT3 was detected in the mitochondria of transformed cells, and was shown to facilitate glycolytic and oxidative phosphorylation activities similar to that of cancer cells (Gough, D.J., et al., Science, 2009, 324, 1713-1716). The inhibition of STAT3 in the mitochondria impaired malignant transformation by activated Ras. The data confirms a Ras-mediated transformation function for STAT3 in the mitochondria in addition to its nuclear roles.

10 Aberrant expression of or constitutive expression of STAT3 is associated with a number of disease processes.

Summary

15 Provided herein are methods, compounds, and compositions for modulating expression of STAT3 mRNA and protein. In certain embodiments, compounds useful for modulating expression of STAT3 mRNA and protein are antisense compounds. In certain embodiments, the antisense compounds are antisense oligonucleotides.

20 In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, STAT3 mRNA levels are reduced. In certain embodiments, STAT3 protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

25 Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such diseases, disorders, and conditions are hyperproliferative diseases, disorders, and conditions. In certain embodiments such hyperproliferative diseases, disorders, and conditions include cancer as well as associated malignancies and metastases. In certain embodiments, such cancers include lung cancer, including non small cell lung cancer (NSCLC), pancreatic cancer, colorectal cancer, multiple myeloma, hepatocellular carcinoma (HCC), glioblastoma, ovarian cancer, osteosarcoma, head and neck cancer, breast cancer, epidermoid carcinomas, intestinal adenomas, prostate cancer, and gastric cancer.

30 Such diseases, disorders, and conditions can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of a hyperproliferative disease include growing older; tobacco use; exposure to sunlight and ionizing radiation; contact with certain chemicals; infection with certain viruses and bacteria; certain hormone therapies; family history of cancer; alcohol use; and certain lifestyle choices including poor diet, lack of physical activity, and/or being overweight. Certain symptoms
35 and outcomes associated with development of a hyperproliferative disease include a thickening or lump in the

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breast or any other part of the body; a new mole or a change in an existing mole; a sore that does not heal; hoarseness or a cough that does not go away; changes in bowel or bladder habits; discomfort after eating; difficulty in swallowing; unexplained weight gain or loss; unusual bleeding or discharge; fatigue; metastasis of one or more tumors throughout the body; cardiovascular complications, including, cardiac arrest and stroke; and death.

In certain embodiments, methods of treatment include administering a STAT3 antisense compound to an individual in need thereof. In certain embodiments, methods of treatment include administering a STAT3 antisense oligonucleotide to an individual in need thereof.

10 Detailed Description

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

Definitions

Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents, applications, published applications and other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

Unless otherwise indicated, the following terms have the following meanings:

“2’-deoxynucleoside” means a nucleoside comprising 2’-H furanosyl sugar moiety, as found naturally occurring in deoxyribonucleosides (DNA). In certain embodiments, a 2’-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (e.g., uracil).

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“2’-O-methoxyethyl” (also 2’-MOE and 2’-O(CH₂)₂-OCH₃) refers to an O-methoxy-ethyl modification of the 2’ position of a furosyl ring. A 2’-O-methoxyethyl modified sugar is a modified sugar.

“2’-MOE nucleoside” (also 2’-O-methoxyethyl nucleoside) means a nucleoside comprising a 2’-MOE modified sugar moiety.

5 “2’-substituted nucleoside” means a nucleoside comprising a substituent at the 2’-position other than H or OH. Unless otherwise indicated, a 2’-substituted nucleoside is not a bicyclic nucleoside.

“5’-methylcytosine” means a cytosine modified with a methyl group attached to the 5’ position. A 5-methylcytosine is a modified nucleobase.

10 “About” means within $\pm 10\%$ of a value. For example, if it is stated, “the compounds affected at least about 70% inhibition of STAT3”, it is implied that the STAT3 levels are inhibited within a range of 63% and 77%.

“Active pharmaceutical agent” means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to STAT3 is an active pharmaceutical agent.

15 “Active target region” or “target region” means a region to which one or more active antisense compounds is targeted. “Active antisense compounds” means antisense compounds that reduce target nucleic acid levels or protein levels.

20 “Administered concomitantly” refers to the co-administration of two agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both agents need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive.

“Administering” means providing a pharmaceutical agent to an individual, and includes, but is not limited to administering by a medical professional and self-administering.

25 “Amelioration” refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

“Animal” refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

30 “Antibody” refers to a molecule characterized by reacting specifically with an antigen in some way, where the antibody and the antigen are each defined in terms of the other. Antibody may refer to a complete antibody molecule or any fragment or region thereof, such as the heavy chain, the light chain, Fab region, and Fc region.

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“Antisense activity” means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

5 “Antisense compound” means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding. Examples of antisense compounds include single-stranded and double-stranded compounds, such as, antisense oligonucleotides, siRNAs, shRNAs, snoRNAs, miRNAs, and satellite repeats.

10 “Antisense inhibition” means reduction of target nucleic acid levels or target protein levels in the presence of an antisense compound complementary to a target nucleic acid as compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

“Antisense oligonucleotide” means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

“Bicyclic sugar” means a furosyl ring modified by the bridging of two atoms. A bicyclic sugar is a modified sugar.

15 “Bicyclic nucleoside” (also BNA) means a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring.

“Cap structure” or “terminal cap moiety” means chemical modifications, which have been incorporated at either terminus of an antisense compound.

20 “cEt” or “constrained ethyl” means a bicyclic nucleoside having a sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-CH(CH₃)-O-2'.

“Constrained ethyl nucleoside” (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge.

25 “Chemically distinct region” refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

“Chimeric antisense compound” means an antisense compound that has at least two chemically distinct regions.

30 “Co-administration” means administration of two or more pharmaceutical agents to an individual. The two or more pharmaceutical agents may be in a single pharmaceutical composition, or may be in separate pharmaceutical compositions. Each of the two or more pharmaceutical agents may be administered through the same or different routes of administration. Co-administration encompasses parallel or sequential administration.

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“Complementarity” means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

“Contiguous nucleobases” means nucleobases immediately adjacent to each other.

“Diluent” means an ingredient in a composition that lacks pharmacological activity, but is
5 pharmaceutically necessary or desirable. For example, the diluent in an injected composition may be a liquid, e.g. saline solution.

“Dose” means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in one, two, or more boluses, tablets, or injections. For example, in certain embodiments where subcutaneous administration is desired, the
10 desired dose requires a volume not easily accommodated by a single injection, therefore, two or more injections may be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week, or month.

“Effective amount” means the amount of active pharmaceutical agent sufficient to effectuate a
15 desired physiological outcome in an individual in need of the agent. The effective amount may vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual’s medical condition, and other relevant factors.

“Fully complementary” or “100% complementary” means each nucleobase of a first nucleic acid has
20 a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

“Gapmer” means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more
25 nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the “gap” and the external regions may be referred to as the “wings.”

“Gap-widened” means a chimeric antisense compound having a gap segment of 12 or more contiguous 2’-deoxyribonucleosides positioned between and immediately adjacent to 5’ and 3’ wing
segments having from one to six nucleosides.

“Hybridization” means the annealing of complementary nucleic acid molecules. In certain
30 embodiments, complementary nucleic acid molecules include an antisense compound and a target nucleic acid.

“Hyperproliferative disease” means a disease characterized by rapid or excessive growth and reproduction of cells. Examples of hyperproliferative diseases include cancer, e.g., carcinomas, sarcomas,
35 lymphomas, and leukemias as well as associated malignancies and metastases.

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“Identifying an animal at risk for hyperproliferative disease” means identifying an animal having been diagnosed with a hyperproliferative disease or identifying an animal predisposed to develop a hyperproliferative disease. Individuals predisposed to develop a hyperproliferative disease include those having one or more risk factors for hyperproliferative disease including older age; history of other
5 hyperproliferative diseases; history of tobacco use; history of exposure to sunlight and/or ionizing radiation; prior contact with certain chemicals, especially continuous contact; past or current infection with certain viruses and bacteria; prior or current use of certain hormone therapies; genetic predisposition; alcohol use; and certain lifestyle choices including poor diet, lack of physical activity, and/or being overweight. Such identification may be accomplished by any method including evaluating an individual’s medical history and
10 standard clinical tests or assessments.

“Immediately adjacent” means there are no intervening elements between the immediately adjacent elements.

“Inhibiting STAT3” means reducing expression of STAT3 mRNA and/or protein levels in the presence of a STAT3 antisense compound, including a STAT3 antisense oligonucleotide, as compared to
15 expression of STAT3 mRNA and/or protein levels in the absence of a STAT3 antisense compound, such as an antisense oligonucleotide.

“Individual” means a human or non-human animal selected for treatment or therapy.

“Internucleoside linkage” refers to the chemical bond between nucleosides.

“Linked nucleosides” means adjacent nucleosides which are bonded together.

20 “Mismatch” or “non-complementary nucleobase” refers to the case when a nucleobase of a first nucleic acid is not capable of pairing with the corresponding nucleobase of a second or target nucleic acid.

“Modified internucleoside linkage” refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

25 “Modified nucleobase” refers to any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An “unmodified nucleobase” means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U).

“Modified nucleotide” means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase. A “modified nucleoside” means a nucleoside having, independently, a modified sugar moiety or modified nucleobase.

30 “Modified oligonucleotide” means an oligonucleotide comprising a modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

“Modified sugar” refers to a substitution or change from a natural sugar.

“Motif” means the pattern of chemically distinct regions in an antisense compound.

“Naturally occurring internucleoside linkage” means a 3' to 5' phosphodiester linkage.

35 “Natural sugar moiety” means a sugar found in DNA (2'-H) or RNA (2'-OH).

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“Nucleic acid” refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA).

“Nucleobase” means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

5 “Nucleobase sequence” means the order of contiguous nucleobases independent of any sugar, linkage, or nucleobase modification.

“Nucleoside” means a nucleobase linked to a sugar.

10 “Nucleoside mimetic” includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics, *e.g.*, non furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to
15 indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system.

“Nucleotide” means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

20 “Off-target effect” refers to an unwanted or deleterious biological effect associated with modulation of RNA or protein expression of a gene other than the intended target nucleic acid.

“Oligomeric compound” or “oligomer” means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

25 “Oligonucleotide” means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

“Parenteral administration” means administration through injection (*e.g.*, bolus injection) or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, *e.g.*, intrathecal or intracerebroventricular administration.

30 “Peptide” means a molecule formed by linking at least two amino acids by amide bonds. Peptide refers to polypeptides and proteins.

“Pharmaceutical composition” means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition may comprise one or more active pharmaceutical agents and a sterile aqueous solution. In certain embodiments, a pharmaceutical composition shows activity
35 in free uptake assay in certain cell lines.

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“Pharmaceutically acceptable derivative” encompasses pharmaceutically acceptable salts, conjugates, prodrugs or isomers of the compounds described herein.

5 “Pharmaceutically acceptable salts” means physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

“Phosphorothioate linkage” means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage (P=S) is a modified internucleoside linkage.

10 “Portion” means a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

“Prevent” refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to indefinitely. Prevent also means reducing risk of developing a disease, disorder, or condition.

15 “Prodrug” means a therapeutic agent that is prepared in an inactive form that is converted to an active form within the body or cells thereof by the action of endogenous enzymes or other chemicals or conditions.

“Side effects” means physiological responses attributable to a treatment other than the desired effects. In certain embodiments, side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

20 “Signal Transducer and Activator of Transcription 3 nucleic acid” or “STAT3 nucleic acid” means any nucleic acid encoding STAT3. For example, in certain embodiments, a STAT3 nucleic acid includes a DNA sequence encoding STAT3, an RNA sequence transcribed from DNA encoding STAT3 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding STAT3. “STAT3 mRNA” means an mRNA encoding a STAT3 protein.

“Single-stranded oligonucleotide” means an oligonucleotide which is not hybridized to a complementary strand.

30 “Specifically hybridizable” refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays and therapeutic treatments.

“Targeting” or “targeted” means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

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“Target nucleic acid,” “target RNA,” “target mRNA,” and “target RNA transcript” all refer to a nucleic acid capable of being targeted by antisense compounds.

“Target segment” means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. “5’ target site” refers to the 5’-most nucleotide of a target segment. “3’ target site” refers to the 3’-most nucleotide of a target segment.

“Therapeutically effective amount” means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

“Treat” refers to administering a pharmaceutical composition to effect an alteration or improvement of a disease, disorder, or condition.

“Unmodified nucleotide” means a nucleotide composed of naturally occurring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. β -D-ribonucleosides) or a DNA nucleotide (i.e. β -D-deoxyribonucleoside).

Certain Embodiments

In certain embodiments provided are methods, compounds, and compositions for inhibiting STAT3 mRNA or protein expression.

In certain embodiments provided are methods for preventing tumor growth and tumor volume. In certain embodiments provided are methods for reducing tumor growth and tumor volume.

In certain embodiments provided are methods, compounds, and compositions for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with STAT3 in an individual in need thereof. Also contemplated are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with STAT3. STAT3 associated diseases, disorders, and conditions include hyperproliferative diseases, *e.g.*, cancer, carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases.

In certain embodiments provided are STAT3 antisense compounds for use in treating, preventing, or ameliorating a STAT3 associated disease. In certain embodiments, STAT3 antisense compounds are STAT3 antisense oligonucleotides, which are capable of inhibiting the expression of STAT3 mRNA and/or STAT3 protein in a cell, tissue, or animal.

In certain embodiments provided are a STAT3 antisense compound as described herein for use in treating or preventing lung cancer, including non small cell lung cancer (NSCLC), pancreatic cancer, colorectal cancer, multiple myeloma, hepatocellular carcinoma (HCC), glioblastoma, ovarian cancer, osteosarcoma, head and neck cancer, breast cancer, epidermoid carcinomas, intestinal adenomas, prostate cancer, and gastric cancer.

In certain embodiments provided are a STAT3 antisense compound as described herein for use in treating or preventing cancer from metastasizing.

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In certain embodiments provided are a STAT3 antisense compound, as described herein, for use in treating, preventing, or ameliorating hyperproliferative diseases, *e.g.*, cancer, carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases.

In certain embodiments provided are antisense compounds targeted to a STAT3 nucleic acid. In certain embodiments, the STAT3 nucleic acid is any of the sequences set forth in GENBANK Accession No. NM_139276.2 (incorporated herein as SEQ ID NO: 1) or the complement of GENBANK Accession No. NT_010755.14 truncated from nucleotides 4185000 to 4264000 (incorporated herein as SEQ ID NO: 2).

In certain embodiments, the antisense compounds provided herein are targeted to any one of the following regions of SEQ ID NO 1: 250-286; 250-285; 264-285; 264-282; 728-745; 729-745; 729-744; 787-803; 867-883; 955-978; 1146-1170; 1896-1920; 1899-1920; 1899-1919; 1899-1918; 1899-1916; 1901-1916; 1946-1963; 1947-1963; 2155-2205; 2155-2187; 2156-2179; 2204-2221; 2681-2696; 2699-2716; 3001-3033; 3008-3033, 3010-3033, 3010-3032, 3015-3033, 3015-3032, 3015-3031, 3016-3033, 3016-3032, 3016-3033; 3452-3499; 3460-3476; 3583-3608; 3591-3616; 3595-3615; 3595-3614; 3595-3612; 3675-3706; 3713-3790; 3715-3735; 3833-3878; 3889-3932; 3977-4012; 4067-4100; 4225-4256; 4234-4252; 4235-4252; 4235-4251; 4236-4252; 4306-4341; 4431-4456; 4439-4454; 4471-4510; 4488-4505; 4530-4558; 4539-4572; 4541-4558; 4636-4801; 4782-4796; 4800-4823; 4811-4847; 4813-4859; 4813-4815; 4813-4831; 4827-4859; 4827-4844; 4842-4859.

In certain embodiments, the antisense compounds provided herein are complementary within any one of the following regions of SEQ ID NO 1: 250-286; 250-285; 264-285; 264-282; 728-745; 729-745; 729-744; 787-803; 867-883; 955-978; 1146-1170; 1896-1920; 1899-1920; 1899-1919; 1899-1918; 1899-1916; 1901-1916; 1946-1963; 1947-1963; 2155-2205; 2155-2187; 2156-2179; 2204-2221; 2681-2696; 2699-2716; 3001-3033; 3008-3033, 3010-3033, 3010-3032, 3015-3033, 3015-3032, 3015-3031, 3016-3033, 3016-3032, 3016-3033; 3452-3499; 3460-3476; 3583-3608; 3591-3616; 3595-3615; 3595-3614; 3595-3612; 3675-3706; 3713-3790; 3715-3735; 3833-3878; 3889-3932; 3977-4012; 4067-4100; 4225-4256; 4234-4252; 4235-4252; 4235-4251; 4236-4252; 4306-4341; 4431-4456; 4439-4454; 4471-4510; 4488-4505; 4530-4558; 4539-4572; 4541-4558; 4636-4801; 4782-4796; 4800-4823; 4811-4847; 4813-4859; 4813-4815; 4813-4831; 4827-4859; 4827-4844; 4842-4859.

In certain embodiments, provided are compounds comprising:
a modified antisense oligonucleotide consisting of 12 to 22 linked nucleosides, wherein the modified

antisense oligonucleotide comprises:

a 5'-wing consisting of 1 to 5 linked nucleosides;

a 3'-wing consisting of 1 to 5 linked nucleosides;

a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and

wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or 2'-substituted nucleoside;

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wherein the nucleobase sequence of the modified antisense oligonucleotide is complementary to an equal length portion of any of nucleobases 250-286; 250-285; 264-285; 264-282; 728-745; 729-745; 729-744; 787-803; 867-883; 955-978; 1146-1170; 1896-1920; 1899-1920; 1899-1919; 1899-1918; 1899-1916; 1901-1916; 1946-1963; 1947-1963; 2155-2205; 2155-2187; 2156-2179; 2204-2221; 2681-2696; 2699-2716; 3001-3033; 3008-3033, 3010-3033, 3010-3032, 3015-3033, 3015-3032, 3015-3031, 3016-3033, 3016-3032, 3016-3033; 3452-3499; 3460-3476; 3583-3608; 3591-3616; 3595-3615; 3595-3614; 3595-3612; 3675-3706; 3713-3790; 3715-3735; 3833-3878; 3889-3932; 3977-4012; 4067-4100; 4225-4256; 4234-4252; 4235-4252; 4235-4251; 4236-4252; 4306-4341; 4431-4456; 4439-4454; 4471-4510; 4488-4505; 4530-4558; 4539-4572; 4541-4558; 4636-4801; 4782-4796; 4800-4823; 4811-4847; 4813-4859; 4813-4815; 4813-4831; 4827-4859; 4827-4844; 4842-4859 of the nucleobase sequence of SEQ ID NO: 1.

In certain embodiments, the antisense compounds provided herein are targeted to any one of the following regions of SEQ ID NO 2: 2668-2688; 2703-2720; 5000-5021; 5001-5017; 5697-5722; 5699-5716; 6475 - 6490; 6475 - 6491; 6476 - 6491; 7682-7705; 8078-8097; 8079-8095; 9862-9811; 9870-9897; 9875-9893; 9875-9891; 9877-9893; 11699-11719; 12342-12366; 12345-12364; 12346-12364; 12347-12364; 12353-12380; 12357-12376; 12358-12376; 12358-12373; 12360-12376; 14128-14148; 16863-16883; 46091-46111; 50692-50709; 50693-50709; 50693-50708; 61325-61349; 66133-66157; 66136-66157; 66136 - 66155; 66136-66153; 66138-66153; 66184-66200; 67067-67083; 4171-74220; 74199-74220; 74202-74220; 74171-74219; 74199-74219; 74202-74219; 74171-74218; 74199-74218; 74202-74218; 74723-74768; 74764-74803; 74782-74802; 74782-74801; 74782-74800; 74782-74799; 74783-74802; 74783-74801; 74783-74800; 74783-74799; 74862-74893; 74900-74977; 74902-74922; 74902-74920; 75070-75119; 75164-75199; 75254-75287; 75412-75443; 75421-75439; 75422-75439; 75422-75438; 75423-75439; 75423-75438; 75493-75528; 75616-75643; 75626-75641; 75658-75699; 75676-75692; 75717-75745; 75726-75759; 75726-75745; 75727-75745; 75728-75745; 75831-75988; 75852-75969; 75969-75984; 75987-76056; 76000-76046; 76000-76032; 76000-76018; 76014-76046; 76014-76032; 76029-76046; and 76031-76046.

In certain embodiments, the antisense compounds provided herein are complementary within any one of the following regions of SEQ ID NO 2: 2668-2688; 2703-2720; 5000-5021; 5001-5017; 5697-5722; 5699-5716; 6475 - 6490; 6475 - 6491; 6476 - 6491; 7682-7705; 8078-8097; 8079-8095; 9862-9811; 9870-9897; 9875-9893; 9875-9891; 9877-9893; 11699-11719; 12342-12366; 12345-12364; 12346-12364; 12347-12364; 12353-12380; 12357-12376; 12358-12376; 12358-12373; 12360-12376; 14128-14148; 16863-16883; 46091-46111; 50692-50709; 50693-50709; 50693-50708; 61325-61349; 66133-66157; 66136-66157; 66136 - 66155; 66136-66153; 66138-66153; 66184-66200; 67067-67083; 4171-74220; 74199-74220; 74202-74220; 74171-74219; 74199-74219; 74202-74219; 74171-74218; 74199-74218; 74202-74218; 74723-74768; 74764-74803; 74782-74802; 74782-74801; 74782-74800; 74782-74799; 74783-74802; 74783-74801; 74783-74800; 74783-74799; 74862-74893; 74900-74977; 74902-74922; 74902-74920; 75070-75119; 75164-75199;

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75254-75287; 75412-75443; 75421-75439; 75422-75439; 75422-75438; 75423-75439; 75423-75438; 75493-75528; 75616-75643; 75626-75641; 75658-75699; 75676-75692; 75717-75745; 75726-75759; 75726-75745; 75727-75745; 75728-75745; 75831-75988; 75852-75969; 75969-75984; 75987-76056; 76000-76046; 76000-76032; 76000-76018; 76014-76046; 76014-76032; 76029-76046; and 76031-76046.

5 In certain embodiments, provided are compounds comprising:

a modified antisense oligonucleotide consisting of 12 to 22 linked nucleosides, wherein the modified antisense oligonucleotide comprises:

a 5'-wing consisting of 1 to 5 linked nucleosides;

a 3'-wing consisting of 1 to 5 linked nucleosides;

10 a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or 2'-substituted nucleoside;

wherein the nucleobase sequence of the modified antisense oligonucleotide is complementary to an equal length portion of any of nucleobases 2668-2688; 2703-2720; 5000-5021; 5001-5017; 5697-5722;

15 5699-5716; 6475 - 6490; 6475 - 6491; 6476 - 6491; 7682-7705; 8078-8097; 8079-8095; 9862-9811; 9870-9897; 9875-9893; 9875-9891; 9877-9893; 11699-11719; 12342-12366; 12345-12364; 12346-12364; 12347-12364; 12353-12380; 12357-12376; 12358-12376; 12358-12373; 12360-12376; 14128-14148; 16863-16883; 46091-46111; 50692-50709; 50693-50709; 50693-50708; 61325-61349; 66133-66157; 66136-66157; 66136 - 66155; 66136-66153; 66138-66153; 66184-66200; 20 67067-67083; 4171-74220; 74199-74220; 74202-74220; 74171-74219; 74199-74219; 74202-74219; 74171-74218; 74199-74218; 74202-74218; 74723-74768; 74764-74803; 74782-74802; 74782-74801; 74782-74800; 74782-74799; 74783-74802; 74783-74801; 74783-74800; 74783-74799; 74862-74893; 74900-74977; 74902-74922; 74902-74920; 75070-75119; 75164-75199; 75254-75287; 75412-75443; 75421-75439; 75422-75439; 75422-75438; 75423-75439; 75423-75438; 25 75493-75528; 75616-75643; 75626-75641; 75658-75699; 75676-75692; 75717-75745; 75726-75759; 75726-75745; 75727-75745; 75728-75745; 75831-75988; 75852-75969; 75969-75984; 75987-76056; 76000-76046; 76000-76032; 76000-76018; 76014-76046; 76014-76032; 76029-76046; and 76031-76046 of the nucleobase sequence of SEQ ID NO: 2.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 12 to 30 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 3008 to 3033 of SEQ ID NO: 1, wherein the nucleobase sequence is complementary to SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous

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nucleobases complementary to an equal length portion of nucleobases 3016 to 3031 of SEQ ID NO: 1, wherein the nucleobase sequence is complementary to SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 6476 to 6491 of SEQ ID NO: 2, wherein the nucleobase sequence is complementary to SEQ ID NO: 2.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 250-286; 250-285; 264-285; 264-282; 728-745; 729-745; 729-744; 787-803; 867-883; 955-978; 1146-1170; 1896-1920; 1899-1920; 1899-1919; 1899-1918; 1899-1916; 1901-1916; 1946-1963; 1947-1963; 2155-2205; 2155-2187; 2156-2179; 2204-2221; 2681-2696; 2699-2716; 3001-3033; 3008-3033, 3010-3033, 3010-3032, 3015-3033, 3015-3032, 3015-3031, 3016-3033, 3016-3032, 3016-3033; 3452-3499; 3460-3476; 3583-3608; 3591-3616; 3595-3615; 3595-3614; 3595-3612; 3675-3706; 3713-3790; 3715-3735; 3833-3878; 3889-3932; 3977-4012; 4067-4100; 4225-4256; 4234-4252; 4235-4252; 4235-4251; 4236-4252; 4306-4341; 4431-4456; 4439-4454; 4471-4510; 4488-4505; 4530-4558; 4539-4572; 4541-4558; 4636-4801; 4782-4796; 4800-4823; 4811-4847; 4813-4859; 4813-4815; 4813-4831; 4827-4859; 4827-4844; or 4842-4859 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 2668-2688; 2703-2720; 5000-5021; 5001-5017; 5697-5722; 5699-5716; 6475 - 6490; 6475 - 6491; 6476 - 6491; 7682-7705; 8078-8097; 8079-8095; 9862-9811; 9870-9897; 9875-9893; 9875-9891; 9877-9893; 11699-11719; 12342-12366; 12345-12364; 12346-12364; 12347-12364; 12353-12380; 12357-12376; 12358-12376; 12358-12373; 12360-12376; 14128-14148; 16863-16883; 46091-46111; 50692-50709; 50693-50709; 50693-50708; 61325-61349; 66133-66157; 66136-66157; 66136 - 66155; 66136-66153; 66138-66153; 66184-66200; 67067-67083; 4171-74220; 74199-74220; 74202-74220; 74171-74219; 74199-74219; 74202-74219; 74171-74218; 74199-74218; 74202-74218; 74723-74768; 74764-74803; 74782-74802; 74782-74801; 74782-74800; 74782-74799; 74783-74802; 74783-74801; 74783-74800; 74783-74799; 74862-74893; 74900-74977; 74902-74922; 74902-74920; 75070-75119; 75164-75199; 75254-75287; 75412-75443; 75421-75439; 75422-75439; 75422-75438; 75423-75439; 75423-75438; 75493-75528; 75616-75643; 75626-75641; 75658-75699; 75676-75692; 75717-75745; 75726-75759; 75726-75745; 75727-75745; 75728-75745; 75831-75988; 75852-75969; 75969-75984; 75987-76056; 76000-76046; 76000-76032; 76000-76018; 76014-76046; 76014-76032; 76029-76046; or

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76031-76046 of SEQ ID NO: 2, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 2.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide comprises the sequence of SEQ ID NO: 245.

5 In certain embodiments, the nucleobase sequence of the modified oligonucleotide consists of the sequence of SEQ ID NO: 245.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide comprises the sequence of SEQ ID NO: 413.

10 In certain embodiments, the nucleobase sequence of the modified oligonucleotide consists of the sequence of SEQ ID NO: 413.

In certain embodiments, the modified oligonucleotide is 100% complementary to SEQ ID NO: 1 or 2.

In certain embodiments, the modified oligonucleotide consists of a single-stranded modified oligonucleotide.

15 In certain embodiments, the modified oligonucleotide has at least one modified internucleoside linkage.

In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, at least one nucleoside comprises a modified sugar.

In certain embodiments, at least one modified sugar is a bicyclic sugar.

In certain embodiments, the bicyclic sugar comprises a 4'-CH₂-O-2' bridge.

20 In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.

In certain embodiments, the modified sugar comprises a 2'-O(CH₂)₂-OCH₃ group.

In certain embodiments, the modified sugar comprises a 2'-O-CH₃ group.

In certain embodiments, at least one nucleoside of the modified oligonucleotide comprises a modified nucleobase.

25 In certain embodiments, the modified nucleobase is a 5'-methylcytosine.

In certain embodiments, the modified oligonucleotide comprises:

a 5'-wing consisting of 1 to 5 linked nucleosides;

a 3'-wing consisting of 1 to 5 linked nucleosides;

a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and

30 wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or one 2'-substituted nucleoside.

In certain embodiments, the modified oligonucleotide comprises:

a 5'-wing consisting of 1 to 5 linked nucleosides;

a 3'-wing consisting of 1 to 5 linked nucleosides;

35 a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and

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wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside and at least one 2'-substituted nucleoside.

In certain embodiments, the 2'-substituted nucleoside comprises any of the group consisting of a 2'-O(CH₂)₂-OCH₃ group or a 2'-O-CH₃ group.

5 In certain embodiments, the bicyclic nucleoside comprises any of the group consisting of a 4'-CH₂-O-2' bridge and a 4'-CH(CH₃)-O-2' bridge.

In certain embodiments, the modified oligonucleotide comprises:

a 5'-wing consisting of 3 linked nucleosides;

a 3'-wing consisting of 3 linked nucleosides;

10 a gap between the 5'-wing and the 3'-wing consisting of 10 linked 2'-deoxynucleosides;

wherein each nucleoside of each of the 5'-wing and the 3'-wing comprises a constrained ethyl nucleoside;

wherein each internucleoside linkage is a phosphorothioate linkage; and

wherein each cytosine is a 5'-methylcytosine.

Certain embodiments provide compounds, comprising a modified oligonucleotide consisting of 12 to
15 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 245.

Certain embodiments provide compounds, comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 413.

20 Certain embodiment provide compounds, comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 9-426, 430-442, 445-464, 471-498, 500-1034, 1036-1512, and 1541-2757.

In certain embodiments, the modified oligonucleotide consists of a single-stranded modified
25 oligonucleotide.

In certain embodiments, at least one internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.

In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, at least one nucleoside comprises a modified sugar.

30 In certain embodiments, at least one modified sugar is a bicyclic sugar.

In certain embodiments, the bicyclic sugar comprises a 4'-CH₂-O-2' bridge.

In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.

In certain embodiments, the modified sugar comprises a 2'-O(CH₂)₂-OCH₃ group.

In certain embodiments, the modified sugar comprises a 2'-O-CH₃ group.

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In certain embodiments, at least one nucleoside of the modified oligonucleotide comprises a modified nucleobase.

In certain embodiments, the modified nucleobase is a 5'-methylcytosine.

In certain embodiments, the modified oligonucleotide comprises:

- 5 a 5'-wing consisting of 1 to 5 linked nucleosides;
- a 3'-wing consisting of 1 to 5 linked nucleosides;
- a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and
- wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or 2'-substituted nucleoside.

10 In certain embodiments, the modified oligonucleotide comprises:

- a 5'-wing consisting of 1 to 5 linked nucleosides;
- a 3'-wing consisting of 1 to 5 linked nucleosides;
- a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and
- wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside and at least one
- 15 2'-substituted nucleoside.

In certain embodiments, the 2'-substituted nucleoside comprises any of the group consisting of a 2'-O(CH₂)₂-OCH₃ group or a 2'-O-CH₃ group.

In certain embodiments, the bicyclic nucleoside comprises any of the group consisting of a 4'-CH₂-O-2' bridge and a 4'-CH(CH₃)-O-2' bridge.

20 In certain embodiments, the modified oligonucleotide comprises:

- a 5'-wing consisting of 3 linked nucleosides;
- a 3'-wing consisting of 3 linked nucleosides;
- a gap between the 5'-wing and the 3'-wing consisting of 10 linked 2'-deoxynucleosides;
- wherein each nucleoside of each of the 5'-wing and the 3'-wing comprises a constrained ethyl nucleoside;
- 25 wherein each internucleoside linkage is a phosphorothioate linkage; and
- wherein each cytosine is a 5'-methylcytosine.

Certain embodiments provide compounds comprising:

a modified oligonucleotide consisting of 12 to 22 linked nucleosides, wherein the modified oligonucleotide comprises:

- 30 a 5'-wing consisting of 1 to 5 linked nucleosides;
- a 3'-wing consisting of 1 to 5 linked nucleosides;
- a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides;
- wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or a 2'-substituted nucleoside;

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wherein the nucleobase sequence of the modified oligonucleotide is complementary to an equal length portion of nucleobases 3016 to 3031 of the nucleobase sequence of SEQ ID NO: 1; and wherein the compound inhibits expression of STAT3 mRNA expression.

Certain embodiments provide compounds comprising:

5 a modified oligonucleotide consisting of 12 to 22 linked nucleosides, wherein the modified oligonucleotide comprises:

a 5'-wing consisting of 1 to 5 linked nucleosides;

a 3'-wing consisting of 1 to 5 linked nucleosides;

a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides;

10 wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or a 2'-substituted nucleoside;

wherein the nucleobase sequence of the modified oligonucleotide is complementary to an equal length portion of nucleobases 6476 to 6491 of the nucleobase sequence of SEQ ID NO: 2; and wherein the compound inhibits expression of STAT3 mRNA expression.

15 In certain embodiments, at least one of the 5'-wing and the 3'-wing comprises at least one 2'-deoxynucleoside.

In certain embodiments, the modified oligonucleotide consists of a single-stranded modified oligonucleotide.

In certain embodiments, the modified oligonucleotide comprises at least one bicyclic nucleoside.

20 In certain embodiments, at least one bicyclic nucleoside comprises a 4'-CH(CH₃)-O-2' bridge.

In certain embodiments, each bicyclic nucleoside comprises a 4'-CH(CH₃)-O-2' bridge.

In certain embodiments, at least one bicyclic nucleoside comprises a 4'-CH₂-O-2' bridge.

In certain embodiments, each bicyclic nucleoside comprises a 4'-CH₂-O-2' bridge.

25 In certain embodiments, the modified oligonucleotide comprises at least one 2'-substituted nucleoside.

In certain embodiments, at least one 2'-substituted nucleoside comprises a 2'-O(CH₂)₂-OCH₃ group.

In certain embodiments, each 2'-substituted nucleoside comprises a 2'-O(CH₂)₂-OCH₃ group.

In certain embodiments, at least one 2'-substituted nucleoside comprises a 2'-O-CH₃ group.

In certain embodiments, each 2'-substituted nucleoside comprises a 2'-O-CH₃ group.

30 In certain embodiments, at least one internucleoside linkage is a modified internucleoside linkage.

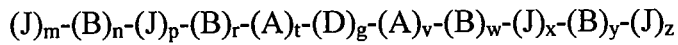
In certain embodiments, each modified internucleoside linkage is a phosphorothioate linkage.

In certain embodiments, at least one nucleoside of the modified oligonucleotide comprises a modified nucleobase.

In certain embodiments, the modified nucleobase is a 5'-methylcytosine.

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In certain embodiments, the modified oligonucleotide has a sugar motif described by Formula A as follows:



wherein:

- 5 each A is independently a 2'-substituted nucleoside;
 each B is independently a bicyclic nucleoside;
 each J is independently either a 2'-substituted nucleoside or a 2'-deoxynucleoside;
 each D is a 2'-deoxynucleoside;
 m is 0-4; n is 0-2; p is 0-2; r is 0-2; t is 0-2; v is 0-2; w is 0-4; x is 0-2; y is 0-2; z is 0-4; g is
 10 6-14;

provided that:

- at least one of m, n, and r is other than 0;
 at least one of w and y is other than 0;
 the sum of m, n, p, r, and t is from 2 to 5; and
 15 the sum of v, w, x, y, and z is from 2 to 5.

In certain embodiments, the modified oligonucleotide has a sugar motif of any of the group consisting of:

- k-d(10)-k
 e-d(10)-k
 20 k-d(10)-e
 k-k-d(10)-k-k
 k-k-d(10)-e-e
 e-e-d(10)-k-k
 k-k-k-d(10)-k-k-k
 25 e-e-e-d(10)-k-k-k
 k-k-k-d(10)-e-e-e
 k-k-k-d(10)-k-k-k
 e-k-k-d(10)-k-k-e
 e-e-k-d(10)-k-k-e
 30 e-d-k-d(10)-k-k-e
 e-k-d(10)-k-e-k-e
 k-d(10)-k-e-k-e-e
 e-e-k-d(10)-k-e-k-e
 e-d-d-k-d(9)-k-k-e

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e-e-e-d(9)-k-k-e

wherein, k is a constrained ethyl nucleoside, e is a 2'-MOE substituted nucleoside, and d is a 2'-deoxynucleoside.

5 Certain embodiments provide methods of treating a hyperproliferative disease in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 9-426, 430-442, 445-464, 471-498, 500-1034, 1036-1512, and 1541-2757.

10 Certain embodiments provide methods of treating a hyperproliferative disease in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 245.

15 Certain embodiments provide methods of treating a hyperproliferative disease in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 413.

In certain embodiments, the administering reduces tumor size in the animal.

In certain embodiments, the administering reduces tumor volume in the animal.

In certain embodiments, the administering prevents metastasis in the animal.

20 In certain embodiments, the administering prolongs survival of the animal.

In certain embodiments, the administering reduces cachaxia in the animal.

25 Certain embodiments provide methods of reducing expression of STAT3 in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 9-426, 430-442, 445-464, 471-498, 500-1034, 1036-1512, and 1541-2757.

30 Certain embodiments provide methods of reducing expression of STAT3 in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 245.

35 Certain embodiments provide methods of reducing expression of STAT3 in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 413.

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In certain embodiments, the compound does not have the wing-gap-wing motif of 2-10-2.

Antisense compounds

Oligomeric compounds include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be “antisense” to a target nucleic acid, meaning that it is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted. In certain such embodiments, an antisense oligonucleotide has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, an antisense compound targeted to a STAT3 nucleic acid is 12 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a STAT3 nucleic acid is 14 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a STAT3 nucleic acid is 12 to 22 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits, 14 to 30 linked subunits, or 12 to 22 linked subunits, respectively. In other embodiments, the antisense compound is 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked subunits. In certain such embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleotides.

In certain embodiments antisense oligonucleotides targeted to a STAT3 nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a STAT3 nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense

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compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

Gautschi et al. (*J. Natl. Cancer Inst.* 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL *in vitro* and *in vivo*. Furthermore, this oligonucleotide demonstrated potent anti-tumor activity *in vivo*.

Maher and Dolnick (*Nuc. Acid. Res.* 16:3341-3358, 1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

In certain embodiments, the compounds as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ of less than 20uM, less than 19uM, less than 18uM, less than 17uM, less than 16uM, less than 15uM, less than 14uM, less than 13uM, less than 12uM, less than 11uM, less than 10uM, less than 9uM, less than 8uM, less than 7uM, less than 6uM, less than 5uM, less than 4uM, less than 3uM, less than 2uM, less than 1uM when delivered to HuVEC cells as described herein.

In certain embodiments, the compounds as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ of less than 1.0uM, less than 0.9uM, less than 0.8uM, less than 0.7uM, less than 0.6uM, less than 0.5uM, less than 0.4uM, less than 0.3uM, less than 0.2uM, less than 0.1uM when delivered to HuVEC cells as described herein.

In certain embodiments, the compounds as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ of less than 0.95uM, less than 0.90uM, less than 0.85uM, less than 0.80uM, less than 0.75uM, less than 0.70uM, less than 0.65uM, less than 0.60uM, less than 0.55uM, less than 0.50uM, less than 0.45uM, less than 0.40uM, less than 0.35uM, less than 0.30uM, less than 0.25uM, less than 0.20uM, less

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than 0.15uM, less than 0.10uM, less than 0.05uM, less than 0.04uM, less than 0.03uM, less than 0.02uM, less than 0.01uM when delivered to HuVEC cells as described herein.

In certain embodiments, the compound as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ of less of less than 20uM, less than 15uM, less than 10uM, less than 5uM, less than 2 uM when delivered by free uptake methods to cancer cell lines as described herein.

In certain embodiments, the compounds as described herein are highly tolerable as demonstrated by having at least one of an increase an ALT or AST value of no more than 4 fold, 3 fold, or 2 fold over saline treated animals or an increase in liver, spleen, or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5%, or 2%. In certain embodiments, the compounds as described herein are highly tolerable as demonstrated by having no increase of ALT or AST over saline treated animals. In certain embodiments, the compounds as described herein are highly tolerable as demonstrated by having no increase in liver, spleen, or kidney weight over saline treated animals. In certain embodiments, these compounds include ISIS 455265, ISIS 455269, ISIS 455271, ISIS 455272, ISIS 455291, ISIS 455371, ISIS 455394, ISIS 455703, ISIS 455429, ISIS 455471, ISIS 455527, ISIS 455530, ISIS 455536, ISIS 455548, ISIS 455611, ISIS 465236, ISIS 465237, ISIS 465588, ISIS 465740, ISIS 465754, ISIS 465830, ISIS 466670, ISIS 466720; ISIS 481374, ISIS 481390, ISIS 481420, ISIS 481431, ISIS 481453, ISIS 481464, ISIS 481475, ISIS 481495, ISIS 481500, ISIS 481501, ISIS 481525, ISIS 481548, ISIS 481549, ISIS 481597, ISIS 481695, ISIS 481700, ISIS 481702, ISIS 481710, ISIS 481725, ISIS 481750, and ISIS 481763. In certain embodiments, such compounds include compounds comprising the sequence of any one of SEQ ID NOs 57, 90, 90, 175, 223, 245, 267, 307, 317, 318, 366, 411, 413, 54, 258, 268, 272, 288, 464, 367, 393, 1564, 1568, 1571, 1572, 1590, 1670, 1693, 1728, 1770, 1826, 1829, 1835, 1847, 1910, 1997, 2168, 2198, 2325, 2339, 2720, 2731, 2732, and 2756.

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to a STAT3 nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned

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between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE and 2'-O-CH₃, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a constrained ethyl). In certain embodiments, wings may include several modified sugar moieties, including, for example 2'-MOE and constrained ethyl. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides, constrained ethyl nucleosides, and 2'-deoxynucleosides.

Each distinct region may comprise uniform sugar moieties, variant, or alternating sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5'-wing, "Y" represents the length of the gap, and "Z" represents the length of the 3'-wing. "X" and "Z" may comprise uniform, variant, or alternating sugar moieties. In certain embodiments, "X" and "Y" may include one or more 2'-deoxynucleosides.

"Y" may comprise 2'-deoxynucleosides. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap is positioned immediately adjacent to each of the 5'-wing and the 3' wing. Thus, no intervening nucleotides exist between the 5'-wing and gap, or the gap and the 3'-wing. Any of the antisense compounds described herein can have a gapmer motif. In certain embodiments, "X" and "Z" are the same, in other embodiments they are different. In certain embodiments, "Y" is between 8 and 15 nucleosides. X, Y, or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleosides.

In certain embodiments, gapmers provided herein include, for example, 11-mers having a motif of 1-9-1.

In certain embodiments, gapmers provided herein include, for example, 12-mers having a motif of 1-9-2, 2-9-1, or 1-10-1.

In certain embodiments, gapmers provided herein include, for example, 13-mers having a motif of 1-9-3, 2-9-2, 3-9-1, 1-10-2, or 2-10-1.

In certain embodiments, gapmers provided herein include, for example, 14-mers having a motif of 1-9-4, 2-9-3, 3-9-2, 4-9-1, 1-10-3, 2-10-2, or 3-10-1.

In certain embodiments, gapmers provided herein include, for example, 15-mers having a motif of 1-9-5, 2-9-4, 3-9-3, 4-9-2, 5-9-1, 1-10-4, 2-10-3, 3-10-2, or 4-10-1.

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In certain embodiments, gapmers provided herein include, for example, 16-mers having a motif of 2-9-5, 3-9-4, 4-9-3, 5-9-2, 1-10-5, 2-10-4, 3-10-3, 4-10-2, or 5-10-1.

In certain embodiments, gapmers provided herein include, for example, 17-mers having a motif of 3-9-5, 4-9-4, 5-9-3, 2-10-5, 3-10-4, 4-10-3, or 5-10-2.

5 In certain embodiments, gapmers provided herein include, for example, 18-mers having a motif of 4-9-5, 5-9-4, 3-10-5, 4-10-4, or 5-10-3.

In certain embodiments, gapmers provided herein include, for example, 19-mers having a motif of 5-9-5, 4-10-5, or 5-10-4.

10 In certain embodiments, gapmers provided herein include, for example, 20-mers having a motif of 5-10-5.

In certain embodiments, the antisense compound has a "wingmer" motif, having a wing-gap or gap-wing configuration, i.e. an X-Y or Y-Z configuration as described above for the gapmer configuration. Thus, wingmer configurations provided herein include, but are not limited to, for example 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, 5-13, 5-8, or 6-8.

15 In certain embodiments, antisense compound targeted to a STAT3 nucleic acid has a 2-10-2 gapmer motif.

In certain embodiments, the antisense compound targeted to a STAT3 nucleic acid has a 3-10-3 gapmer motif.

20 In certain embodiments, the antisense compound targeted to a STAT3 nucleic acid has a 5-10-5 gapmer motif.

In certain embodiments, the antisense compound targeted to a STAT3 nucleic acid has a 1-10-5 gapmer motif.

In certain embodiments, the antisense compound targeted to a STAT3 nucleic acid has a 3-10-4 gapmer motif.

25 In certain embodiments, the antisense compound targeted to a STAT3 nucleic acid has a 2-10-4 gapmer motif.

In certain embodiments, the antisense compound targeted to a STAT3 nucleic acid has a 4-9-3 gapmer motif.

30 In certain embodiments, the antisense compound targeted to a STAT3 nucleic acid has a gap-widened motif.

In certain embodiments, the antisense compounds targeted to a STAT3 nucleic acid has any of the following sugar motifs:

k-d(10)-k

e-d(10)-k

35 k-d(10)-e

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k-k-d(10)-k-k

k-k-d(10)-e-e

e-e-d(10)-k-k

k-k-k-d(10)-k-k-k

5 e-e-e-d(10)-k-k-k

k-k-k-d(10)-e-e-e

k-k-k-d(10)-k-k-k

e-k-k-d(10)-k-k-e

e-e-k-d(10)-k-k-e

10 e-d-k-d(10)-k-k-e

e-k-d(10)-k-e-k-e

k-d(10)-k-e-k-e-e

e-e-k-d(10)-k-e-k-e

15 e-d-d-k-d(9)-k-k-e

e-e-e-e-d(9)-k-k-e

wherein, k is a constrained ethyl nucleoside, e is a 2'-MOE substituted nucleoside, and d is a 2'-deoxynucleoside.

20 In certain embodiments, the antisense oligonucleotide has a sugar motif described by Formula A as follows: $(J)_m-(B)_n-(J)_p-(B)_r-(A)_t-(D)_g-(A)_v-(B)_w-(J)_x-(B)_y-(J)_z$

wherein:

each A is independently a 2'-substituted nucleoside;

each B is independently a bicyclic nucleoside;

each J is independently either a 2'-substituted nucleoside or a 2'-deoxynucleoside;

25 each D is a 2'-deoxynucleoside;

m is 0-4; n is 0-2; p is 0-2; r is 0-2; t is 0-2; v is 0-2; w is 0-4; x is 0-2; y is 0-2; z is 0-4; g is

6-14;

provided that:

at least one of m, n, and r is other than 0;

30 at least one of w and y is other than 0;

the sum of m, n, p, r, and t is from 2 to 5; and

the sum of v, w, x, y, and z is from 2 to 5.

BIOL0142WO*Target Nucleic Acids, Target Regions and Nucleotide Sequences*

Nucleotide sequences that encode STAT3 include, without limitation, the following: GENBANK Accession No. NM_139276.2 (incorporated herein as SEQ ID NO: 1) and the complement of GENBANK Accession No. NT_010755.14 truncated from nucleotides 4185000 to 4264000 (incorporated herein as SEQ ID NO: 2).

It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for STAT3 can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels. In certain embodiments, the desired effect is reduction of levels of protein encoded by the target nucleic acid or a phenotypic change associated with the target nucleic acid.

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain embodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifically exclude a certain structurally defined region such as the start codon or stop codon.

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The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in STAT3 mRNA levels are indicative of inhibition of STAT3 expression. Reductions in levels of a STAT3 protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of STAT3 expression. In certain embodiments, reduced cellular growth, reduced tumor growth, and reduced tumor volume can be indicative of inhibition of STAT3 expression. In certain embodiments, amelioration of symptoms associated with cancer can be indicative of inhibition of STAT3 expression. In certain embodiments, reduction of cachexia is indicative of inhibition of STAT3 expression. In certain embodiments, reduction of cancer markers can be indicative of inhibition of STAT3 expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a STAT3 nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a STAT3 nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a STAT3 nucleic acid).

Non-complementary nucleobases between an antisense compound and a STAT3 nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a STAT3 nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure,

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mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a STAT3 nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having four noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403 410; Zhang and Madden, *Genome Res.*, 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, 1981, 2, 482 489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a STAT3 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, “fully complementary” means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be “fully complementary” to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending

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on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a STAT3 nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a STAT3 nucleic acid, or specified portion thereof.

The antisense compounds provided herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As

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used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, a portion of the antisense oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

BIOL0142WO*Modified Internucleoside Linkages*

The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiester, phosphotriester, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a STAT3 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds provided herein can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring moiety. Examples of chemically modified ribofuranose rings include, without limitation, addition of substituent groups (including 5' and 2' substituent groups); bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA); replacement of the ribosyl ring oxygen atom with S, N(R), or C(R1)(R2) (R = H, C₁-C₁₂ alkyl or a protecting group); and combinations thereof. Examples of chemically modified sugars include, 2'-F-5'-methyl substituted nucleoside (*see*, PCT International Application WO 2008/101157, published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides), replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (*see*, published U.S. Patent Application US2005/0130923, published on June 16, 2005), or, alternatively, 5'-substitution of a BNA (*see*, PCT International Application WO 2007/134181, published on 11/22/07, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include, without limitation, nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH₃, and 2'-O(CH₂)₂OCH₃ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, OCF₃, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(Rm)(Rn), and O-CH₂-C(=O)-N(Rm)(Rn), where each Rm and Rn is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl.

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As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include, without limitation, nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises a 4' to 2' bicyclic nucleoside.

5 Examples of such 4' to 2' bicyclic nucleosides, include, but are not limited to, one of the formulae: 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' and 4'-CH(CH₂OCH₃)-O-2', and analogs thereof (*see*, U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2', and analogs thereof (*see*, published PCT International Application WO2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2', and analogs thereof (*see*, published PCT International Application WO2008/150729, published December 11, 10 2008); 4'-CH₂-O-N(CH₃)-2' (*see*, published U.S. Patent Application US2004/0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (*see*, U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (*see*, Chattopadhyaya, *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2', and analogs thereof (*see*, published PCT International Application WO 2008/154401, published on December 8, 2008). Also see, for example: Singh *et al.*, *Chem. Commun.*, 1998, 15 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630; Wahlestedt *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava *et al.*, *J. Am. Chem. Soc.*, 129(26) 8362-8379 (Jul. 4, 2007); Elayadi *et al.*, *Curr. Opinion Invens. Drugs*, 2001, 2, 558-561; Braasch *et al.*, *Chem. Biol.*, 2001, 8, 1-7; Orum *et al.*, *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; U.S. Patent Nos U.S. 6,670,461, 7,053,207, 20 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 7,399,845; published PCT International applications WO 2004/106356, WO 94/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; and U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Application Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922. Each 25 of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (*see* PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the pentofuranosyl sugar moiety 30 wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from -[C(R_a)(R_b)]_n-, -C(R_a)=C(R_b)-, -C(R_a)=N-, -C(=NR_a)-, -C(=O)-, -C(=S)-, -O-, -Si(R_a)₂-, -S(=O)_x-, and -N(R_a)-;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

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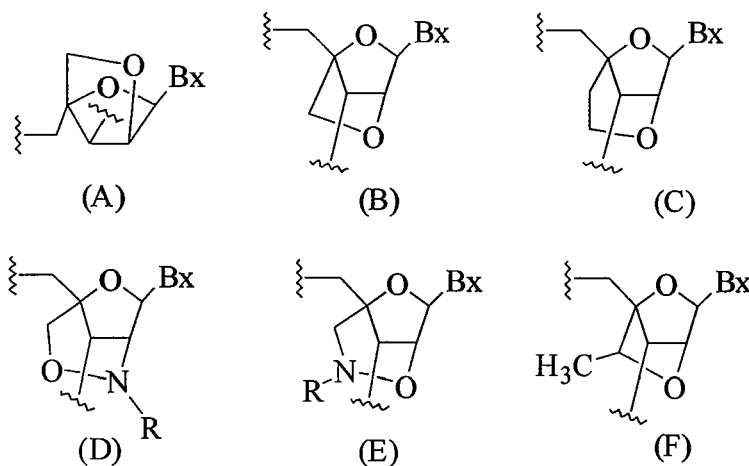
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5 - C_7 alicyclic radical, substituted C_5 - C_7 alicyclic radical, halogen, OJ_1 , NJ_1J_2 , SJ_1 , N_3 , $COOJ_1$, acyl ($C(=O)$ -H), substituted acyl, CN, sulfonyl ($S(=O)_2$ - J_1), or sulfoxyl ($S(=O)$ - J_1); and

each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)$ -H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted C_1 - C_{12} aminoalkyl, or a protecting group.

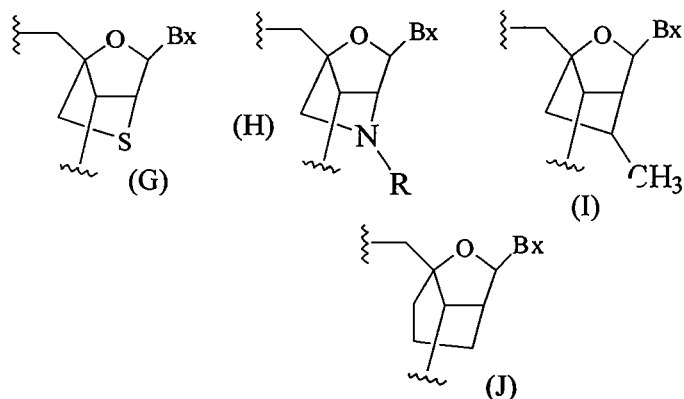
In certain embodiments, the bridge of a bicyclic sugar moiety is, $-[C(R_a)(R_b)]_n$, $-[C(R_a)(R_b)]_n-O$, $-C(R_aR_b)-N(R)-O$ or $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is $4'-CH_2-2'$, $4'-(CH_2)_2-2'$, $4'-(CH_2)_3-2'$, $4'-CH_2-O-2'$, $4'-(CH_2)_2-O-2'$, $4'-CH_2-O-N(R)-2'$, and $4'-CH_2-N(R)-O-2'$, wherein each R is, independently, H, a protecting group, or C_1 - C_{12} alkyl.

In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a $4'-2'$ methylene-oxy bridge, may be in the α -L configuration or in the β -D configuration. Previously, α -L-methyleneoxy ($4'-CH_2-O-2'$) BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden *et al.*, *Nucleic Acids Research*, 2003, 21, 6365-6372).

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α -L-Methyleneoxy ($4'-CH_2-O-2'$) BNA, (B) β -D-Methyleneoxy ($4'-CH_2-O-2'$) BNA, (C) Ethyleneoxy ($4'-(CH_2)_2-O-2'$) BNA, (D) Aminoxy ($4'-CH_2-O-N(R)-2'$) BNA, (E) Oxyamino ($4'-CH_2-N(R)-O-2'$) BNA, (F) Methyl(methyleneoxy) ($4'-CH(CH_3)-O-2'$) BNA, (G) methylene-thio ($4'-CH_2-S-2'$) BNA, (H) methylene-amino ($4'-CH_2-N(R)-2'$) BNA, (I) methyl carbocyclic ($4'-CH_2-CH(CH_3)-2'$) BNA, and (J) propylene carbocyclic ($4'-(CH_2)_3-2'$) BNA as depicted below.

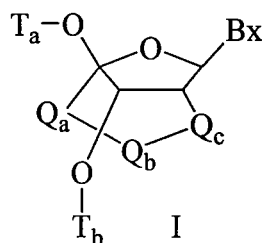


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wherein Bx is the base moiety and R is, independently, H, a protecting group or C₁-C₁₂ alkyl.

In certain embodiments, bicyclic nucleoside having Formula I:



wherein:

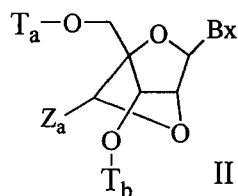
Bx is a heterocyclic base moiety;

-Q_a-Q_b-Q_c- is -CH₂-N(R_c)-CH₂-, -C(=O)-N(R_c)-CH₂-, -CH₂-O-N(R_c)-, -CH₂-N(R_c)-O-, or -N(R_c)-O-CH₂;

R_c is C₁-C₁₂ alkyl or an amino protecting group; and

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium.

In certain embodiments, bicyclic nucleoside having Formula II:



wherein:

Bx is a heterocyclic base moiety;

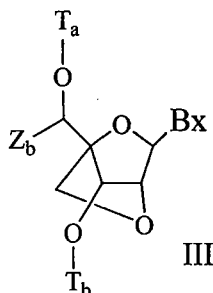
T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

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Z_a is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, substituted C_1 - C_6 alkyl, substituted C_2 - C_6 alkenyl, substituted C_2 - C_6 alkynyl, acyl, substituted acyl, substituted amide, thiol, or substituted thio.

In one embodiment, each of the substituted groups is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c , NJ_cJ_d , SJ_c , N_3 , $OC(=X)J_e$, and $NJ_eC(=X)NJ_cJ_d$, wherein each J_c , J_d , and J_e is, independently, H, C_1 - C_6 alkyl, or substituted C_1 - C_6 alkyl and X is O or NJ_c .

In certain embodiments, bicyclic nucleoside having Formula III:



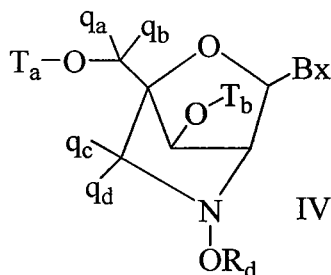
10 wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

15 Z_b is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, substituted C_1 - C_6 alkyl, substituted C_2 - C_6 alkenyl, substituted C_2 - C_6 alkynyl, or substituted acyl ($C(=O)-$).

In certain embodiments, bicyclic nucleoside having Formula IV:



wherein:

20 Bx is a heterocyclic base moiety;

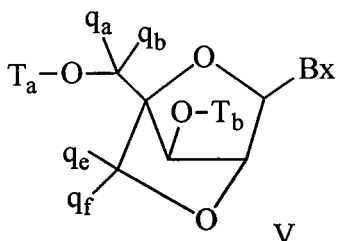
T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

R_d is C_1 - C_6 alkyl, substituted C_1 - C_6 alkyl, C_2 - C_6 alkenyl, substituted C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, or substituted C_2 - C_6 alkynyl;

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each q_a , q_b , q_c and q_d is, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, acyl, substituted acyl, C₁-C₆ aminoalkyl, or substituted C₁-C₆ aminoalkyl;

In certain embodiments, bicyclic nucleoside having Formula V:



wherein:

Bx is a heterocyclic base moiety;

10 T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

q_a , q_b , q_c and q_f are each, independently, hydrogen, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k or N(H)C(=S)NJ_jJ_k;

15 or q_e and q_f together are =C(q_g)(q_h);

q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl, or substituted C₁-C₁₂ alkyl.

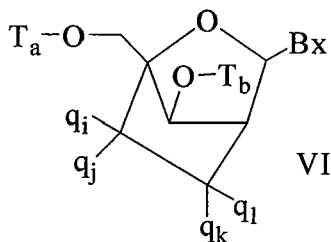
The synthesis and preparation of the methyleneoxy (4'-CH₂-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine, and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (*see, e.g., Koshkin et al., Tetrahedron*, 1998, 54, 3607-3630).

20 BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogues of methyleneoxy (4'-CH₂-O-2') BNA, methyleneoxy (4'-CH₂-O-2') BNA, and 2'-thio-BNAs, have also been prepared (*see, e.g., Kumar et al., Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (*see, e.g., Wengel et al., WO 99/14226*). Furthermore, synthesis of 2'-amino-BNA, a novel conformationally restricted high-affinity oligonucleotide analog, has been described in the art (*see, e.g., Singh et al., J. Org. Chem.*, 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

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In certain embodiments, bicyclic nucleoside having Formula VI:



wherein:

5 Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

each q_i, q_j, q_k and q_l is, independently, H, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-
 10 C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k, or N(H)C(=S)NJ_jJ_k; and

q_i and q_j or q_l and q_k together are =C(q_g)(q_h), wherein q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl, or substituted C₁-C₁₂ alkyl.

One carbocyclic bicyclic nucleoside having a 4'-(CH₂)₃-2' bridge and the alkenyl analog, bridge 4'-
 15 CH=CH-CH₂-2', have been described (*see, e.g., Freier et al., Nucleic Acids Research, 1997, 25(22), 4429-4443* and *Albaek et al., J. Org. Chem., 2006, 71, 7731-7740*). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g., Srivastava et al., J. Am. Chem. Soc. 2007, 129(26), 8362-8379*).

As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic
 20 nucleoside comprising a furanose ring comprising a bridge connecting the 2' carbon atom and the 4' carbon atom.

As used herein, "monocyclic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

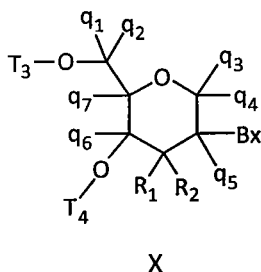
As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain
 25 embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but
 30 not limited to: O[(CH₂)_nO]_mCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, OCH₂C(=O)N(H)CH₃, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. Other 2'- substituent groups can also be

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selected from: C₁-C₁₂ alkyl; substituted alkyl; alkenyl; alkynyl; alkaryl; aralkyl; O-alkaryl or O-aralkyl; SH; SCH₃; OCN; Cl; Br; CN; CF₃; OCF₃; SOCH₃; SO₂CH₃; ONO₂; NO₂; N₃; NH₂; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving pharmacokinetic properties; and a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modified nucleosides comprise a 2'-MOE side chain (*see, e.g.,* Baker et al., *J. Biol. Chem.*, 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'-O-methyl, O-propyl, and O-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (*see, e.g.,* Martin, P., *Helv. Chim. Acta*, 1995, 78, 486-504; Altmann et al., *Chimia*, 1996, 50, 168-176; Altmann et al., *Biochem. Soc. Trans.*, 1996, 24, 630-637; and Altmann et al., *Nucleosides Nucleotides*, 1997, 16, 917-926).

As used herein, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (*see* Leumann, *CJ. Bioorg. & Med. Chem.* (2002) 10:841-854), fluoro HNA (F-HNA), or those compounds having Formula X:

Formula X:



wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula X:

Bx is a heterocyclic base moiety;

T₃ and T₄ are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T₃ and T₄ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl; and

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one of R_1 and R_2 is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ_1J_2 , SJ_1 , N_3 , $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, and CN , wherein X is O , S , or NJ_1 , and each J_1 , J_2 , and J_3 is, independently, H or C_1 - C_6 alkyl.

In certain embodiments, the modified THP nucleosides of Formula X are provided wherein q_m , q_n , q_p , q_r , q_s , q_t , and q_u are each H . In certain embodiments, at least one of q_m , q_n , q_p , q_r , q_s , q_t , and q_u is other than H . In certain embodiments, at least one of q_m , q_n , q_p , q_r , q_s , q_t and q_u is methyl. In certain embodiments, THP nucleosides of Formula X are provided wherein one of R_1 and R_2 is F . In certain embodiments, R_1 is fluoro and R_2 is H , R_1 is methoxy and R_2 is H , and R_1 is methoxyethoxy and R_2 is H .

As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH . 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring and nucleosides with non-bridging 2' substituents, such as allyl, amino, azido, thio, O-allyl, O- C_1 - C_{10} alkyl, $-OCF_3$, O- $(CH_2)_2$ -O- CH_3 , 2'-O $(CH_2)_2$ SCH $_3$, O- $(CH_2)_2$ -O- $N(R_m)(R_n)$, or O- CH_2 -C(=O)- $N(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1 - C_{10} alkyl. 2'-modified nucleosides may further comprise other modifications, for example, at other positions of the sugar and/or at the nucleobase.

As used herein, "2'-F" refers to a sugar comprising a fluoro group at the 2' position.

As used herein, "2'-OMe" or "2'-OCH $_3$ " or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an $-OCH_3$ group at the 2' position of the sugar ring.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (*see, e.g.*, review article: Leumann, J. C, *Bioorganic & Medicinal Chemistry*, **2002**, *10*, 841-854).

Such ring systems can undergo various additional substitutions to enhance activity.

Methods for the preparations of modified sugars are well known to those skilled in the art.

In nucleotides having modified sugar moieties, the nucleobase moieties (natural, modified, or a combination thereof) are maintained for hybridization with an appropriate nucleic acid target.

In certain embodiments, antisense compounds comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a cEt. In certain embodiments, the cEt modified nucleotides are arranged throughout the wings of a gapmer motif.

BIOL0142WO*Compositions and Methods for Formulating Pharmaceutical Compositions*

Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

An antisense compound targeted to a STAT3 nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a STAT3 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

Conjugated Antisense compounds

Antisense compounds may be covalently linked to one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. Typical conjugate groups include cholesterol moieties and lipid moieties. Additional conjugate groups include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-

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cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

5

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of STAT3 nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commercial vendors (e.g. American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HuVEC cells, b.END cells, HepG2 cells, Hep3B cells, and primary hepatocytes.

10

15 *In vitro testing of antisense oligonucleotides*

Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

Cells may be treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

20

One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN (Invitrogen, Carlsbad, CA). Antisense oligonucleotides may be mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

25

Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE in OPTI-MEM 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a LIPOFECTAMINE concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

30

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

Cells are treated with antisense oligonucleotides by routine methods. Cells may be harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein. In general, when treatments are performed in multiple replicates, the data are presented as the average of the replicate treatments.

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The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

Free Uptake Assays

In certain embodiments, transfection-independent activity (i.e., free uptake) of antisense oligonucleotides in cancer cell lines is a measure of potency. Free uptake may be measured in cancer cell lines such as, for example, SK-BR-3 cells, U251-MG cells, MDA-MB-231 cells, H460 cells, A431 cells, colo205 cells, SNB-19 cells, SK-OV3 cells, H1993 lung cancer cells, H358 lung cancer cells, PC-9 lung cancer cells, KHM-35 lung cancer cells, Capan-1 pancreatic cancer cells, HPAF-11 pancreatic cancer cells, and Colo 201 colorectal cancer cells.

In free uptake assays, antisense oligonucleotides are administered to cells lines without the aid of a transfection agent or electroporation. Antisense oligonucleotides are administered to cell lines at one or more doses and percent inhibition of target mRNA or protein expression is measured. Where multiple doses are administered, IC50 may be measured. In certain embodiments, antisense oligonucleotides exhibiting a high degree of potency, as measured by percent inhibition after single dose or multiple doses, are preferred over antisense oligonucleotides exhibiting a lower degree of potency. Those antisense oligonucleotides exhibiting a high degree of *in vitro* potency are more likely to exhibit *in vivo* potency.

RNA Isolation

RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. RNA is prepared using methods well known in the art, for example, using the TRIZOL Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocols.

Analysis of inhibition of target levels or expression

Inhibition of levels or expression of a STAT3 nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitative real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

BIOL0142WO*Quantitative Real-Time PCR Analysis of Target RNA Levels*

Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Methods of quantitative real-time PCR are well known in the art.

5 Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT and real-time PCR reagents may be obtained from Invitrogen (Carlsbad, CA). RT real-time-PCR reactions are carried out by methods well known to those skilled in the art.

10 Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN RNA quantification reagent (Invitrogen, Inc. Eugene, OR). Methods of RNA quantification
15 by RIBOGREEN are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN fluorescence.

Probes and primers are designed to hybridize to a STAT3 nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of STAT3 nucleic acids can be assessed by measuring STAT3 protein levels. Protein levels of STAT3 can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay
25 (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of mouse,
30 rat, monkey, and human STAT3 are commercially available.

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of STAT3 and produce phenotypic changes, such as, reduced cellular growth,
35 amelioration of symptoms associated with cancer, reduction of cachexia, and reduction of cancer markers.

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Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, subcutaneous, intrathecal, and intracerebroventricular. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from liver tissue and changes in STAT3 nucleic acid expression are measured. Changes in STAT3 protein levels are also measured.

In certain embodiments, xenograft tumor models are used to measure the effect of antisense oligonucleotides on tumor growth and metastasis. In xenograft tumor model described herein, cells from a cancerous cell line are inoculated into an animal. Such cell lines may include, for example, human breast cancer cells, MDA-MB-231, A431 human epidermoid carcinoma, U251 human glioma tumor cells, and human NCI-H460 non-small cell lung carcinoma cells. Certain compounds described herein and used in xenograft models described herein may target human STAT3, mouse STAT3, rat STAT3, and/or monkey STAT3. Certain compounds described herein and used in xenograft models described herein may cross-react with one or more species STAT3. In certain embodiments, compounds described herein and used in xenograft models described herein may be more potent inhibitors of tumor growth and tumor volume than the data suggests wherein endogenous STAT3 is not reduced (due to lack of cross-reactivity).

Certain Indications

In certain embodiments, provided are methods, compounds, and compositions of treating an individual comprising administering one or more pharmaceutical compositions provided herein. In certain embodiments, the individual has a hyperproliferative disease. In certain embodiments, the hyperproliferative disease is cancer, e.g., carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases. In certain embodiments, the type of cancer is lung cancer, including non small cell lung cancer (NSCLC), pancreatic cancer, colorectal cancer, multiple myeloma, hepatocellular carcinoma (HCC), glioblastoma, ovarian cancer, osteosarcoma, head and neck cancer, breast cancer, epidermoid carcinomas, intestinal adenomas, prostate cancer, and gastric cancer. In certain embodiments, the individual is at risk for a hyperproliferative disease, including, cancer, e.g., carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases. This includes individuals having one or more risk factors for developing a hyperproliferative disease, including, growing older; tobacco use; exposure to sunlight and ionizing radiation; contact with certain chemicals; infection with certain viruses and bacteria; certain hormone therapies; genetic predisposition; alcohol use; and certain lifestyle choices including poor diet, lack of physical activity, and/or being overweight. In certain embodiments, the individual has been identified as in need of treatment for a hyperproliferative disease. In certain embodiments, are provided methods for

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prophylactically reducing STAT3 expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to a STAT3 nucleic acid.

5 In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing metastasis of a cancer associated with the upregulation of certain genes, such as STAT3, at the tumor bone interface to bone. In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing cancer from metastasizing to bone. In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing renal cell carcinoma, breast cancer, non small cell lung carcinoma, and
10 prostate cancer from metastasizing to bone.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to a STAT3 nucleic acid is accompanied by monitoring of STAT3 levels in the serum of an individual to determine an individual's response to administration of the antisense compound. An individual's response to administration of the antisense compound is used by a physician to determine the
15 amount and duration of therapeutic intervention.

In certain embodiments, administration of an antisense compound targeted to a STAT3 nucleic acid results in reduction of STAT3 expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to a STAT3 nucleic acid results in reduced cellular growth, reduced tumor
20 growth, reduced tumor volume, amelioration of symptoms associated with cancer, and reduction of cancer markers. In certain embodiments, administration of a STAT3 antisense compound decreases cellular growth, tumor growth, and tumor volume by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to
25 STAT3 are used for the preparation of a medicament for treating a patient suffering or susceptible to a hyperproliferative disease.

Certain Combination Therapies

In certain embodiments, one or more pharmaceutical compositions provided herein are co-
30 administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease, disorder, or condition as the one or more pharmaceutical compositions provided herein. In certain embodiments, such one or more other pharmaceutical agents are designed to treat a different disease, disorder, or condition as the one or more pharmaceutical compositions provided herein. In certain embodiments, such one or more other
35 pharmaceutical agents are designed to treat an undesired side effect of one or more pharmaceutical

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compositions provided herein. In certain embodiments, one or more pharmaceutical compositions provided herein are co-administered with another pharmaceutical agent to treat an undesired effect of that other pharmaceutical agent. In certain embodiments, one or more pharmaceutical compositions provided herein are co-administered with another pharmaceutical agent to produce a combinational effect. In certain
5 embodiments, one or more pharmaceutical compositions provided herein are co-administered with another pharmaceutical agent to produce a synergistic effect.

In certain embodiments, one or more pharmaceutical compositions provided herein and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions provided herein and one or more other pharmaceutical agents are administered
10 at different times. In certain embodiments, one or more pharmaceutical compositions provided herein and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions provided herein and one or more other pharmaceutical agents are prepared separately. In certain embodiments, one or more other pharmaceutical agents include all-trans retinoic acid, azacitidine, azathioprine, bleomycin, carboplatin, capecitabine,
15 cisplatin, chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabine, hydroxyurea, idarubicin, imatinib, mechlorethamine, mercaptopurine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, valrubicin, vinblastine, vincristine, vindesine, or vinorelbine. In certain embodiments, one or more other pharmaceutical agents include another antisense oligonucleotide. In certain embodiments,
20 another antisense oligonucleotide is a second STAT3 antisense oligonucleotide.

In certain embodiments, one or more other pharmaceutical agents include molecular targeted therapies. In certain embodiments, the molecular targeted therapy is an EGFR inhibitor, a mTOR inhibitor, a HER2 inhibitor, or a VEGF/VEGFR inhibitor. In certain embodiments, EGFR inhibitors include gefitinib, erlotinib, lapatinib, cetuximab, panitumumab. In certain embodiments, mTOR inhibitors include everolimus
25 and temsirolimus. In certain embodiments, HER2 inhibitors include trastuzumab and lapatinib. In certain embodiments, VEGF/VEGFR inhibitors include pazopanib, bevacizumab, sunitinib, and sorafenib.

In certain embodiments, one more pharmaceutical compositions provided herein are administered with radiation therapy. In certain embodiments, one or more pharmaceutical compositions are administered at the same time as radiation therapy. In certain embodiments, one or more pharmaceutical compositions are
30 administered before radiation therapy. In certain embodiments, one or more pharmaceutical compositions are administered after radiation therapy. In certain embodiments, one or more pharmaceutical compositions are administered at various time points throughout a radiation therapy regimen.

In certain embodiments, radiation therapy is useful for inhibiting tumor growth. In certain embodiments, radiation therapy is useful for increasing overall survival. In certain embodiments, radiation
35 therapy used in conjunction with administration of one or more pharmaceuticals provided herein is

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advantageous over using either therapy alone because both radiation therapy and administration with one or more pharmaceuticals can be limited to achieve effective antiproliferative response with limited toxicity.

In certain embodiments, a physician designs a therapy regimen including both radiation therapy and administration of one more pharmaceutical compositions provided herein. In certain embodiments, a
5 physician designs a therapy regimen including radiation therapy, administration of one or more pharmaceutical compositions provided herein, and administration of one or more other chemotherapeutic agents.

Tolerability

10 In certain embodiments, the compounds provided herein display minimal side effects. Side effects include responses to the administration of the antisense compound that are typically unrelated to the targeting of STAT3, such as an inflammatory response in the animal. In certain embodiments compounds are well tolerated by the animal. Increased tolerability can depend on a number of factors, including, but not limited to, the nucleotide sequence of the antisense compound,
15 chemical modifications to the nucleotides, the particular motif of unmodified and modified nucleosides in the antisense compound, or combinations thereof. Tolerability may be determined by a number of factors. Such factors include body weight, organ weight, liver function, kidney function, platelet count, white blood cell count.

In certain embodiments, the compounds provided herein demonstrate minimal effect on
20 organ weight. In certain embodiments, the compounds demonstrate less than a 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, 2-fold or no significant increase in spleen and/or liver weight.

In certain embodiments, the compounds provided herein demonstrate minimal effect on liver function. Factors for the evaluation of liver function include ALT levels, AST levels, plasma bilirubin levels and plasma albumin levels. In certain embodiments the compounds provided herein
25 demonstrate less than a 7-fold, less than a 6-fold, less than a 5-fold, less than a 4-fold, less than a 3-fold or less than a 2-fold or no significant increase in ALT or AST. In certain embodiments the compounds provided herein demonstrate less than a 3-fold, less than a 2-fold or no significant increase in plasma bilirubin levels.

In certain embodiments, the compounds provided herein demonstrate minimal effect on
30 kidney function. In certain embodiments, the compounds provided herein demonstrate less than a 3-fold, less than a 2-fold, or no significant increase in plasma concentrations of blood urea nitrogen

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(BUN). In certain embodiments, the compounds provided herein demonstrate less than a 6-fold, 5-fold, 4-fold, 3-fold, 2-fold, or no significant increase in the ratio of urine protein to creatinine.

In certain embodiments, the compounds provided herein demonstrate minimal effect on hematological factors. In certain embodiments, the compounds provided herein demonstrate less than a 60%, 50%, 40%, 30%, 20%, 10% or 5% decrease in platelet count. In certain embodiments, the compounds provided herein demonstrate less than a 4-fold, less than a 3-fold, less than a 2-fold or no significant increase in monocyte count.

In certain embodiments compounds further display favorable pharmacokinetics. In certain embodiments, antisense compounds exhibit relatively high half-lives in relevant biological fluids or tissues.

In certain embodiments, compounds or compositions further display favorable viscosity. In certain embodiments, the viscosity of the compound or composition is no more than 40cP at a concentration of 165-185 mg/mL.

In other embodiments, the compounds display combinations of the characteristics above and reduce STAT3 mRNA expression in an animal model with high efficiency.

EXAMPLES*Non-limiting disclosure and incorporation by reference*

While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references recited in the present application is incorporated herein by reference in its entirety.

Example 1: Antisense inhibition of human STAT3 in HuVEC cells

Antisense oligonucleotides were designed targeting a human STAT3 nucleic acid and were tested for their effect on human STAT3 mRNA expression *in vitro*. The chimeric antisense oligonucleotides presented in Tables 1 and 2 were designed as either 2-10-2 cEt gapmers or 3-10-3 cEt gapmers. The 2-10-2 cEt gapmers are 14 nucleotides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising two nucleosides each. The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three

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nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5'-methylcytosines.

Potency of cEt gapmers was compared to ISIS 337332, ISIS 337333, and ISIS 345785, which are 5-10-5 MOE gapmers targeting human STAT3 and are further described in USPN 7,307,069, incorporated herein by reference.

Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS199 (forward sequence ACATGCCACTTTGGTGTTCATAA, designated herein as SEQ ID NO: 6; reverse sequence TCTTCGTAGATTGTGCTGATAGAGAAC, designated herein as SEQ ID NO: 7; probe sequence CAGTATAGCCGCTTCCTGCAAGAGTCGAA, designated herein as SEQ ID NO: 8) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells. All cEt gapmers and MOE gapmers were tested under the same conditions.

"Human Target start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Human Target stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Table 1 is targeted to human STAT3 mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_139276.2). Each gapmer listed in Table 2 is targeted to the human STAT3 genomic sequence, designated herein as SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_010755.14 truncated from nucleotides 4185000 to 4264000).

Table 1

Inhibition of human STAT3 mRNA levels by cEt and MOE chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

ISIS NO	Human Start Site	Human Stop Site	Sequence	Motif	Wing Chem	% inhibition	SEQ ID NO
481350	76	91	TCCAGGATCCGGTTGG	3-10-3	cEt	52	9
481575	77	90	CCAGGATCCGGTTG	2-10-2	cEt	41	10
481351	132	147	GGCCGAAGGGCCTCTC	3-10-3	cEt	14	11
481576	133	146	GCCGAAGGGCCTCT	2-10-2	cEt	8	12
481352	225	240	CCTGCTAAAATCAGGG	3-10-3	cEt	15	13
481577	226	239	CTGCTAAAATCAGG	2-10-2	cEt	12	14
481353	240	255	ATTCCATTGGGCCATC	3-10-3	cEt	78	15
481578	241	254	TTCCATTGGGCCAT	2-10-2	cEt	51	16

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481354	264	279	CCGTGTGTCAAGCTGC	3-10-3	cEt	98	17
481579	265	278	CGTGTGTCAAGCTG	2-10-2	cEt	91	18
481355	322	337	ACTGCCGCAGCTCCAT	3-10-3	cEt	95	19
481580	323	336	CTGCCGCAGCTCCA	2-10-2	cEt	76	20
481356	346	361	GACTCTCAATCCAAGG	3-10-3	cEt	83	21
481581	347	360	ACTCTCAATCCAAG	2-10-2	cEt	31	22
481357	375	390	TTCTTTGCTGGCCGCA	3-10-3	cEt	97	23
481582	376	389	TCTTTGCTGGCCGC	2-10-2	cEt	87	24
481358	403	418	GATTATGAAACACCAA	3-10-3	cEt	85	25
481583	404	417	ATTATGAAACACCA	2-10-2	cEt	20	26
481359	429	444	ATACTGCTGGTCAATC	3-10-3	cEt	90	27
481584	430	443	TACTGCTGGTCAAT	2-10-2	cEt	42	28
481360	459	474	GAGAACATTCGACTCT	3-10-3	cEt	75	29
481585	460	473	AGAACATTCGACTC	2-10-2	cEt	77	30
481361	474	489	TAGATTGTGCTGATAG	3-10-3	cEt	90	31
481586	475	488	AGATTGTGCTGATA	2-10-2	cEt	81	32
481362	490	505	ACTGCTTGATTCTTCG	3-10-3	cEt	59	33
481587	491	504	CTGCTTGATTCTTC	2-10-2	cEt	23	34
481363	511	526	CAAGATACCTGCTCTG	3-10-3	cEt	84	35
481588	512	525	AAGATACCTGCTCT	2-10-2	cEt	58	36
481364	542	557	GCCACAATCCGGGCAA	3-10-3	cEt	36	37
481589	543	556	CCACAATCCGGGCA	2-10-2	cEt	69	38
481365	589	604	CAGTGGCTGCAGTCTG	3-10-3	cEt	36	39
481590	590	603	AGTGGCTGCAGTCT	2-10-2	cEt	30	40
481366	607	622	GGCCCCCTTGCTGGGC	3-10-3	cEt	1	41
481591	608	621	GCCCCCTTGCTGGG	2-10-2	cEt	0	42
481367	638	653	GTCACCACGGCTGCTG	3-10-3	cEt	70	43
481592	639	652	TCACCACGGCTGCT	2-10-2	cEt	48	44
481368	659	674	TCCAGCATCTGCTGCT	3-10-3	cEt	81	45
481593	660	673	CCAGCATCTGCTGC	2-10-2	cEt	46	46
481369	675	690	ATCCTGAAGGTGCTGC	3-10-3	cEt	29	47
481594	676	689	TCCTGAAGGTGCTG	2-10-2	cEt	16	48
481370	701	716	TCTAGATCCTGCACTC	3-10-3	cEt	79	49
481595	702	715	CTAGATCCTGCACT	2-10-2	cEt	47	50
481371	709	724	TTTTCTGTTCTAGATC	3-10-3	cEt	83	51
481596	710	723	TTTCTGTTCTAGAT	2-10-2	cEt	48	52
481372	730	745	GGAGATTCTCTACCAC	3-10-3	cEt	85	53
481597	731	744	GAGATTCTCTACCA	2-10-2	cEt	80	54
481373	751	766	AGTTGAAATCAAAGTC	3-10-3	cEt	87	55
481598	752	765	GTTGAAATCAAAGT	2-10-2	cEt	6	56
481374	788	803	AGATCTTGCATGTCTC	3-10-3	cEt	92	57
481599	789	802	GATCTTGCATGTCT	2-10-2	cEt	51	58

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481375	799	814	TGTTTCCATTTCAGATC	3-10-3	cEt	65	59
481600	800	813	GTTTCCATTTCAGAT	2-10-2	cEt	42	60
481376	868	883	TCCGCATCTGGTCCAG	3-10-3	cEt	82	61
481601	869	882	CCGCATCTGGTCCA	2-10-2	cEt	70	62
481785	872	885	TCTCCGCATCTGGT	2-10-2	cEt	28	63
481377	884	899	TCACTCACGATGCTTC	3-10-3	cEt	85	64
481602	885	898	CACTCACGATGCTT	2-10-2	cEt	55	65
481378	892	907	CCGCCAGCTCACTCAC	3-10-3	cEt	89	66
481603	893	906	CGCCAGCTCACTCA	2-10-2	cEt	60	67
481379	955	970	TCCAGTCAGCCAGCTC	3-10-3	cEt	91	68
481604	956	969	CCAGTCAGCCAGCT	2-10-2	cEt	70	69
481380	963	978	CCGCCTCTTCCAGTCA	3-10-3	cEt	73	70
481605	964	977	CGCCTCTTCCAGTC	2-10-2	cEt	55	71
481381	1010	1025	CGATCTAGGCAGATGT	3-10-3	cEt	26	72
481606	1011	1024	GATCTAGGCAGATG	2-10-2	cEt	35	73
481382	1045	1060	GAGATTCTGCTAATGA	3-10-3	cEt	81	74
481607	1046	1059	AGATTCTGCTAATG	2-10-2	cEt	51	75
481383	1053	1068	CTGAAGTTGAGATTCT	3-10-3	cEt	84	76
481608	1054	1067	TGAAGTTGAGATTC	2-10-2	cEt	26	77
481384	1098	1113	AACTTTTTGCTGCAAC	3-10-3	cEt	76	78
481609	1099	1112	ACTTTTTGCTGCAA	2-10-2	cEt	34	79
481385	1113	1128	GTCCCCTTTGTAGGAA	3-10-3	cEt	41	80
481610	1114	1127	TCCCCTTTGTAGGA	2-10-2	cEt	37	81
481386	1186	1201	AGGCACTTTTCATTAA	3-10-3	cEt	45	82
481611	1187	1200	GGCACTTTTCATTA	2-10-2	cEt	32	83
481387	1225	1240	CAGGATGCATGGGCAT	3-10-3	cEt	92	84
481612	1226	1239	AGGATGCATGGGCA	2-10-2	cEt	86	85
481388	1269	1284	TTAGTAGTGAAGTGG	3-10-3	cEt	74	86
481613	1270	1283	TTAGTAGTGAAGTGG	2-10-2	cEt	22	87
481389	1282	1297	CCAGCAACCTGACTTT	3-10-3	cEt	66	88
481614	1283	1296	CAGCAACCTGACTT	2-10-2	cEt	34	89
481390	1305	1320	ATAATTCAACTCAGGG	3-10-3	cEt	92	90
481615	1306	1319	TAATTCAACTCAGG	2-10-2	cEt	48	91
481391	1314	1329	TTTAAGCTGATAATTC	3-10-3	cEt	44	92
481616	1315	1328	TTAAGCTGATAATT	2-10-2	cEt	0	93
481392	1326	1341	GCACACTTTAATTTTA	3-10-3	cEt	49	94
481617	1327	1340	CACACTTTAATTTT	2-10-2	cEt	1	95
481393	1347	1362	GTCCCCAGAGTCTTTG	3-10-3	cEt	39	96
481618	1348	1361	TCCCCAGAGTCTTT	2-10-2	cEt	41	97
481394	1437	1452	GAGGCTGCCGTTGTTG	3-10-3	cEt	62	98
481619	1438	1451	AGGCTGCCGTTGTT	2-10-2	cEt	29	99
481395	1468	1483	CCCTCAGGGTCAAGTG	3-10-3	cEt	72	100

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481620	1469	1482	CCTCAGGGTCAAGT	2-10-2	cEt	37	101
481396	1480	1495	CACATCTCTGCTCCCT	3-10-3	cEt	92	102
481621	1481	1494	ACATCTCTGCTCCC	2-10-2	cEt	74	103
481397	1517	1532	ATCAGGGAAGCATCAC	3-10-3	cEt	59	104
481622	1518	1531	TCAGGGAAGCATCA	2-10-2	cEt	49	105
481398	1542	1557	GATCAGGTGCAGCTCC	3-10-3	cEt	73	106
481623	1543	1556	ATCAGGTGCAGCTC	2-10-2	cEt	40	107
481399	1563	1578	ATACACCTCGGTCTCA	3-10-3	cEt	73	108
481624	1564	1577	TACACCTCGGTCTC	2-10-2	cEt	43	109
481400	1579	1594	TCTTGAGGCCTTGGTG	3-10-3	cEt	47	110
481625	1580	1593	CTTGAGGCCTTGGT	2-10-2	cEt	16	111
481401	1589	1604	TCTAGGTCAATCTTGA	3-10-3	cEt	74	112
481626	1590	1603	CTAGGTCAATCTTG	2-10-2	cEt	54	113
481402	1599	1614	GGAGTGGGTCTCTAGG	3-10-3	cEt	52	114
481627	1600	1613	GAGTGGGTCTCTAG	2-10-2	cEt	13	115
481789	1604	1617	CAAGGAGTGGGTCT	2-10-2	cEt	10	116
481403	1607	1622	ACTGGCAAGGAGTGGG	3-10-3	cEt	58	117
481628	1608	1621	CTGGCAAGGAGTGG	2-10-2	cEt	38	118
481404	1633	1648	TCTGACAGATGTTGGA	3-10-3	cEt	50	119
481629	1634	1647	CTGACAGATGTTGG	2-10-2	cEt	64	120
481405	1641	1656	ATTTGGCATCTGACAG	3-10-3	cEt	75	121
481630	1642	1655	TTTGGCATCTGACA	2-10-2	cEt	39	122
481406	1691	1706	TTCTTGGGATTGTTGG	3-10-3	cEt	72	123
481631	1692	1705	TCCTTGGGATTGTTG	2-10-2	cEt	33	124
481407	1729	1744	CCCAGGTTCCAATTGG	3-10-3	cEt	50	125
481632	1730	1743	CCAGGTTCCAATTG	2-10-2	cEt	32	126
481408	1780	1795	CTCGCTTGGTGGTGGA	3-10-3	cEt	53	127
481633	1781	1794	TCGCTTGGTGGTGG	2-10-2	cEt	35	128
481409	1795	1810	GCTCGATGCTCAGTCC	3-10-3	cEt	86	129
481634	1796	1809	CTCGATGCTCAGTC	2-10-2	cEt	43	130
481410	1825	1840	CCAAGAGTTTCTCTGC	3-10-3	cEt	91	131
481635	1826	1839	CAAGAGTTTCTCTG	2-10-2	cEt	43	132
481411	1840	1855	AATTCACACCAGGTCC	3-10-3	cEt	72	133
481636	1841	1854	ATTCACACCAGGTC	2-10-2	cEt	42	134
481412	1858	1873	TGATCTGACACCCTGA	3-10-3	cEt	90	135
481637	1859	1872	GATCTGACACCCTG	2-10-2	cEt	79	136
481413	1866	1881	AGCCCATGTGATCTGA	3-10-3	cEt	80	137
481638	1867	1880	GCCCATGTGATCTG	2-10-2	cEt	64	138
481414	1888	1903	CCATGTTTTCTTTGCA	3-10-3	cEt	69	139
481639	1889	1902	CATGTTTTCTTTGC	2-10-2	cEt	16	140
481415	1896	1911	CTTGCCAGCCATGTTT	3-10-3	cEt	88	141
481640	1897	1910	TTGCCAGCCATGTT	2-10-2	cEt	57	142

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337332	1898	1917	GAAGCCCTTGCCAGCCATGT	5-10-5	MOE	63	143
481416	1901	1916	AAGCCCTTGCCAGCCA	3-10-3	cEt	87	144
481641	1902	1915	AGCCCTTGCCAGCC	2-10-2	cEt	68	145
337333	1903	1922	AAGGAGAAGCCCTTGCCAGC	5-10-5	MOE	49	146
481417	1903	1918	AGAAGCCCTTGCCAGC	3-10-3	cEt	97	147
481418	1904	1919	GAGAAGCCCTTGCCAG	3-10-3	cEt	92	148
481642	1904	1917	GAAGCCCTTGCCAG	2-10-2	cEt	67	149
481419	1905	1920	GGAGAAGCCCTTGCCA	3-10-3	cEt	83	150
481643	1905	1918	AGAAGCCCTTGCCA	2-10-2	cEt	58	151
481644	1906	1919	GAGAAGCCCTTGCC	2-10-2	cEt	45	152
481420	1948	1963	ACTTTTTCACAAGGTC	3-10-3	cEt	94	153
481645	1949	1962	CTTTTTCACAAGGT	2-10-2	cEt	50	154
481421	2021	2036	CTCAAGATGGCCCGCT	3-10-3	cEt	86	155
481646	2022	2035	TCAAGATGGCCCGC	2-10-2	cEt	41	156
481422	2036	2051	CCTGGAGGCTTAGTGC	3-10-3	cEt	80	157
481647	2037	2050	CTGGAGGCTTAGTG	2-10-2	cEt	0	158
481423	2077	2092	CTCCTTCTTTGCTGCT	3-10-3	cEt	69	159
481648	2078	2091	TCCTTCTTTGCTGC	2-10-2	cEt	51	160
481424	2093	2108	CAAGTGAAAGTGACGC	3-10-3	cEt	70	161
481649	2094	2107	AAGTGAAAGTGACG	2-10-2	cEt	25	162
481425	2115	2130	ACCGCTGATGTCCTTC	3-10-3	cEt	78	163
481650	2116	2129	CCGCTGATGTCCTT	2-10-2	cEt	79	164
481426	2131	2146	ACTGGATCTGGGTCTT	3-10-3	cEt	80	165
481651	2132	2145	CTGGATCTGGGTCT	2-10-2	cEt	64	166
481427	2155	2170	GCTGCTTTGTGTATGG	3-10-3	cEt	75	167
481652	2156	2169	CTGCTTTGTGTATG	2-10-2	cEt	82	168
481428	2164	2179	TGTTTCAGCTGCTGCTT	3-10-3	cEt	77	169
481653	2165	2178	GTTCAGCTGCTGCT	2-10-2	cEt	79	170
481429	2172	2187	TGACATGTTGTTTCAGC	3-10-3	cEt	84	171
481654	2173	2186	GACATGTTGTTTCAG	2-10-2	cEt	70	172
481430	2190	2205	CATGATGATTTTCAGCA	3-10-3	cEt	67	173
481655	2191	2204	ATGATGATTTTCAGC	2-10-2	cEt	31	174
481431	2206	2221	CCATGATCTTATAGCC	3-10-3	cEt	91	175
481656	2207	2220	CATGATCTTATAGC	2-10-2	cEt	0	176
481432	2233	2248	GTGGAGACACCAGGAT	3-10-3	cEt	55	177
481657	2234	2247	TGGAGACACCAGGA	2-10-2	cEt	58	178
481433	2256	2271	AATGTCAGGATAGAGA	3-10-3	cEt	73	179
481658	2257	2270	ATGTCAGGATAGAG	2-10-2	cEt	62	180
481434	2266	2281	CCTCCTTGGAATGTC	3-10-3	cEt	73	181
345785	2267	2286	TGCCTCCTCCTTGGAATGT	5-10-5	MOE	50	182
481659	2267	2280	CTCCTTGGAATGT	2-10-2	cEt	51	183
481435	2269	2284	CCTCCTCCTTGGAAT	3-10-3	cEt	49	184

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481660	2270	2283	CTCCTCCTTGGGAA	2-10-2	cEt	54	185
481436	2275	2290	CGAATGCCTCCTCCTT	3-10-3	cEt	82	186
481661	2276	2289	GAATGCCTCCTCCT	2-10-2	cEt	76	187
481437	2296	2311	TCTCTGGCCGACAATA	3-10-3	cEt	49	188
481662	2297	2310	CTCTGGCCGACAAT	2-10-2	cEt	43	189
481438	2353	2368	ACTTGGTCTTCAGGTA	3-10-3	cEt	51	190
481663	2354	2367	CTTGGTCTTCAGGT	2-10-2	cEt	52	191
481439	2371	2386	TTGGTGTACACAGAT	3-10-3	cEt	82	192
481664	2372	2385	TGGTGTACACAGA	2-10-2	cEt	89	193
481440	2387	2402	GTATTGCTGCAGGTCG	3-10-3	cEt	79	194
481665	2388	2401	TATTGCTGCAGGTC	2-10-2	cEt	43	195
481441	2395	2410	GGTCAATGGTATTGCT	3-10-3	cEt	55	196
481666	2396	2409	GTCAATGGTATTGC	2-10-2	cEt	36	197
481442	2403	2418	CATCGGCAGGTCAATG	3-10-3	cEt	44	198
481667	2404	2417	ATCGGCAGGTCAAT	2-10-2	cEt	31	199
481443	2423	2438	GAATCTAAAGTGCGGG	3-10-3	cEt	78	200
481668	2424	2437	AATCTAAAGTGCGG	2-10-2	cEt	41	201
481444	2431	2446	GCATCAATGAATCTAA	3-10-3	cEt	66	202
481669	2432	2445	CATCAATGAATCTA	2-10-2	cEt	0	203
481445	2439	2454	TCCAAACTGCATCAAT	3-10-3	cEt	70	204
481670	2440	2453	CCAAACTGCATCAA	2-10-2	cEt	60	205
481446	2460	2475	TTCAGCACCTTCACCA	3-10-3	cEt	44	206
481671	2461	2474	TCAGCACCTTCACC	2-10-2	cEt	41	207
481447	2476	2491	GCCCTCCTGCTGAGGG	3-10-3	cEt	10	208
481672	2477	2490	CCCTCCTGCTGAGG	2-10-2	cEt	15	209
481448	2484	2499	CTCAAACCTGCCCTCCT	3-10-3	cEt	29	210
481797	2484	2497	CAAACCTGCCCTCCT	2-10-2	cEt	11	211
481673	2485	2498	TCAAACCTGCCCTCC	2-10-2	cEt	33	212
481449	2503	2518	CCATGTCAAAGGTGAG	3-10-3	cEt	77	213
481674	2504	2517	CATGTCAAAGGTGA	2-10-2	cEt	31	214
481450	2530	2545	GGGAGGTAGCGCACTC	3-10-3	cEt	53	215
481675	2531	2544	GGAGGTAGCGCACT	2-10-2	cEt	41	216
481451	2592	2607	GAATGCAGGTAGGCGC	3-10-3	cEt	55	217
481676	2593	2606	AATGCAGGTAGGCG	2-10-2	cEt	39	218
481452	2631	2646	TTTCAGATGATCTGGG	3-10-3	cEt	71	219
481677	2632	2645	TTCAGATGATCTGG	2-10-2	cEt	38	220
481574	2650	2665	GGAACCACAAAGTTAG	3-10-3	cEt	69	221
481799	2651	2664	GAACCACAAAGTTA	2-10-2	cEt	50	222
481453	2681	2696	GATAGCAGAAGTAGGA	3-10-3	cEt	92	223
481678	2682	2695	ATAGCAGAAGTAGG	2-10-2	cEt	78	224
481454	2702	2717	AAAGTGCCCAGATTGC	3-10-3	cEt	85	225
481679	2703	2716	AAGTGCCCAGATTG	2-10-2	cEt	69	226

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481455	2722	2737	CACTCATTTCTCTATT	3-10-3	cEt	74	227
481680	2723	2736	ACTCATTTCTCTAT	2-10-2	cEt	39	228
481456	2767	2782	AACACATCCTTATTTG	3-10-3	cEt	48	229
481681	2768	2781	ACACATCCTTATTT	2-10-2	cEt	47	230
481457	2779	2794	TGGGTCTCAGAGAACA	3-10-3	cEt	88	231
481682	2780	2793	GGGTCTCAGAGAAC	2-10-2	cEt	77	232
481458	2832	2847	CAAGACATTTCCTTTT	3-10-3	cEt	54	233
481683	2833	2846	AAGACATTTCCTTT	2-10-2	cEt	29	234
481459	2908	2923	GGAGGCACTTGTCTAA	3-10-3	cEt	76	235
481684	2909	2922	GAGGCACTTGTCTA	2-10-2	cEt	89	236
481460	2943	2958	TTACAGAAACAGGCAG	3-10-3	cEt	83	237
481685	2944	2957	TACAGAAACAGGCA	2-10-2	cEt	36	238
481461	2969	2984	AGCTATAGGTGGCCTG	3-10-3	cEt	75	239
481686	2970	2983	GCTATAGGTGGCCT	2-10-2	cEt	70	240
481462	2984	2999	ATGCCAGGAGTATGTA	3-10-3	cEt	89	241
481687	2985	2998	TGCCAGGAGTATGT	2-10-2	cEt	80	242
481463	3001	3016	CAAGGTAAAAAGTGC	3-10-3	cEt	88	243
481688	3002	3015	AAGGTAAAAAGTG	2-10-2	cEt	13	244
481464	3016	3031	CTATTTGGATGTCAGC	3-10-3	cEt	97	245
481689	3017	3030	TATTTGGATGTCAG	2-10-2	cEt	40	246
481465	3032	3047	TAGATAGTCCTATCTT	3-10-3	cEt	51	247
481690	3033	3046	AGATAGTCCTATCT	2-10-2	cEt	64	248
481466	3047	3062	AAGAAACCTAGGGCTT	3-10-3	cEt	74	249
481691	3048	3061	AGAAACCTAGGGCT	2-10-2	cEt	77	250
481467	3097	3112	GCTGATACAGTGTTTT	3-10-3	cEt	74	251
481692	3098	3111	CTGATACAGTGTTT	2-10-2	cEt	74	252
481468	3112	3127	ATACAGAAAGGCTATG	3-10-3	cEt	71	253
481693	3113	3126	TACAGAAAGGCTAT	2-10-2	cEt	25	254
481469	3127	3142	GCTTAAGTTTCTTAAA	3-10-3	cEt	61	255
481694	3128	3141	CTTAAGTTTCTTAA	2-10-2	cEt	0	256
481470	3461	3476	AGCACCAAGGAGGCTG	3-10-3	cEt	49	257
481695	3462	3475	GCACCAAGGAGGCT	2-10-2	cEt	83	258
481471	3476	3491	AAGCTGAATGCTTAAA	3-10-3	cEt	36	259
481696	3477	3490	AGCTGAATGCTTAA	2-10-2	cEt	33	260
481472	3491	3506	TTACCAGCCTGAAGGA	3-10-3	cEt	76	261
481697	3492	3505	TACCAGCCTGAAGG	2-10-2	cEt	63	262
481473	3506	3521	CAGGGATTATATAAAT	3-10-3	cEt	53	263
481698	3507	3520	AGGGATTATATAAA	2-10-2	cEt	15	264
481474	3521	3536	ACCTGAAGCCCGTTTC	3-10-3	cEt	80	265
481699	3522	3535	CCTGAAGCCCGTTT	2-10-2	cEt	57	266
481475	3536	3551	TGTCTTAAGGGTTTGA	3-10-3	cEt	93	267
481700	3537	3550	GTCTTAAGGGTTTG	2-10-2	cEt	89	268

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481476	3551	3566	GGTTGCAGCTTCAGAT	3-10-3	cEt	92	269
481701	3552	3565	GTTGCAGCTTCAGA	2-10-2	cEt	60	270
481477	3567	3582	TCAACACCAAAGGCCA	3-10-3	cEt	95	271
481702	3568	3581	CAACACCAAAGGCC	2-10-2	cEt	89	272
481478	3585	3600	TCCTTAAACCTTCCTA	3-10-3	cEt	84	273
481703	3586	3599	CCTTAAACCTTCCT	2-10-2	cEt	57	274
481479	3600	3615	AAAATGCTTAGATTCT	3-10-3	cEt	80	275
481704	3601	3614	AAATGCTTAGATTC	2-10-2	cEt	32	276
481480	3628	3643	AAATAAGTCTATTTAT	3-10-3	cEt	5	277
481705	3629	3642	AATAAGTCTATTTA	2-10-2	cEt	25	278
481481	3648	3663	GGCCAATACATTACAA	3-10-3	cEt	63	279
481706	3649	3662	GCCAATACATTACA	2-10-2	cEt	56	280
481482	3670	3685	TGCCCAGCCTTACTCA	3-10-3	cEt	55	281
481707	3671	3684	GCCCAGCCTTACTC	2-10-2	cEt	43	282
481483	3685	3700	GTTGTAAGCACCTCT	3-10-3	cEt	1	283
481708	3686	3699	TTGTAAGCACCTC	2-10-2	cEt	56	284
481484	3700	3715	AGAAAGGGAGTCAAGG	3-10-3	cEt	60	285
481709	3701	3714	GAAAGGGAGTCAAG	2-10-2	cEt	27	286
481485	3717	3732	GCAGATCAAGTCCAGG	3-10-3	cEt	90	287
481710	3718	3731	CAGATCAAGTCCAG	2-10-2	cEt	88	288
481486	3730	3745	AGCCTCTGAAACAGCA	3-10-3	cEt	75	289
481711	3731	3744	GCCTCTGAAACAGC	2-10-2	cEt	74	290
481487	3746	3761	CCCACAGAAACAACCT	3-10-3	cEt	66	291
481712	3747	3760	CCACAGAAACAACC	2-10-2	cEt	45	292
481488	3761	3776	AGCCCTGATAAGGCAC	3-10-3	cEt	23	293
481713	3762	3775	GCCCTGATAAGGCA	2-10-2	cEt	18	294
481489	3776	3791	AATCAGAAGTATCCCA	3-10-3	cEt	60	295
481714	3777	3790	ATCAGAAGTATCCC	2-10-2	cEt	43	296
481490	3833	3848	GCCTCTAGCAGGATCA	3-10-3	cEt	78	297
481715	3834	3847	CCTCTAGCAGGATC	2-10-2	cEt	79	298
481491	3848	3863	CACGCAAGGAGACATG	3-10-3	cEt	70	299
481716	3849	3862	ACGCAAGGAGACAT	2-10-2	cEt	68	300
481492	3863	3878	TGAGGGACCTTTAGAC	3-10-3	cEt	61	301
481717	3864	3877	GAGGGACCTTTAGA	2-10-2	cEt	44	302
481493	3886	3901	CAGGATTCCTAAAACA	3-10-3	cEt	43	303
481718	3887	3900	AGGATTCCTAAAAC	2-10-2	cEt	7	304
481494	3901	3916	ATGAGGTCCTGAGACC	3-10-3	cEt	60	305
481719	3902	3915	TGAGGTCCTGAGAC	2-10-2	cEt	29	306
481495	3940	3955	CATCATGTCCAACCTG	3-10-3	cEt	92	307
481720	3941	3954	ATCATGTCCAACCT	2-10-2	cEt	63	308
481496	3955	3970	GGGCCCCATAGTGTGC	3-10-3	cEt	29	309
481721	3956	3969	GGCCCCATAGTGTG	2-10-2	cEt	19	310

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481497	3977	3992	AGCTCAACCAGACACG	3-10-3	cEt	67	311
481722	3978	3991	GCTCAACCAGACAC	2-10-2	cEt	69	312
481498	3992	4007	GAACCATATTCCTGA	3-10-3	cEt	90	313
481723	3993	4006	AACCATATTCCTG	2-10-2	cEt	49	314
481499	4007	4022	CAAGAACTGGCTAAG	3-10-3	cEt	43	315
481724	4008	4021	AAGAACTGGCTAA	2-10-2	cEt	17	316
481500	4022	4037	GCCACTGGATATCACC	3-10-3	cEt	92	317
481501	4048	4063	AACTGAATGAAGACGC	3-10-3	cEt	91	318
481726	4049	4062	ACTGAATGAAGACG	2-10-2	cEt	56	319
481502	4063	4078	CCTTTGCCCTGCATGA	3-10-3	cEt	85	320
481727	4064	4077	CTTTGCCCTGCATG	2-10-2	cEt	70	321
481503	4078	4093	AAGTTTATCAGTAAGC	3-10-3	cEt	57	322
481728	4079	4092	AGTTTATCAGTAAG	2-10-2	cEt	22	323
481504	4093	4108	TACGAGGGCAGACTCA	3-10-3	cEt	60	324
481729	4094	4107	ACGAGGGCAGACTC	2-10-2	cEt	22	325
481505	4108	4123	AGGTATACACCCTCAT	3-10-3	cEt	45	326
481730	4109	4122	GGTATACACCCTCA	2-10-2	cEt	47	327
481506	4123	4138	CCTCAGAGGGAGGCCA	3-10-3	cEt	32	328
481731	4124	4137	CTCAGAGGGAGGCC	2-10-2	cEt	0	329
481507	4138	4153	GGGAGGAGTCACCAGC	3-10-3	cEt	64	330
481732	4139	4152	GGAGGAGTCACCAG	2-10-2	cEt	59	331
481508	4205	4220	TAGCCAGCCAAGGCGG	3-10-3	cEt	33	332
481733	4206	4219	AGCCAGCCAAGGCG	2-10-2	cEt	50	333
481509	4220	4235	ACAGGAGAGGCGAGCT	3-10-3	cEt	46	334
481734	4221	4234	CAGGAGAGGCGAGC	2-10-2	cEt	28	335
481510	4237	4252	TAGGTGTTCCCATACG	3-10-3	cEt	95	336
481735	4238	4251	AGGTGTTCCCATAC	2-10-2	cEt	22	337
481511	4258	4273	GGCAGCCCATCCAGCA	3-10-3	cEt	43	338
481736	4259	4272	GCAGCCCATCCAGC	2-10-2	cEt	54	339
481512	4275	4290	CATGCCTCTGAGTCAG	3-10-3	cEt	30	340
481737	4276	4289	ATGCCTCTGAGTCA	2-10-2	cEt	31	341
481513	4290	4305	GTTGCCAAATCCGGCC	3-10-3	cEt	85	342
481738	4291	4304	TTGCCAAATCCGGC	2-10-2	cEt	70	343
481514	4305	4320	GCAAGGTGGTTTTGAG	3-10-3	cEt	85	344
481739	4306	4319	CAAGGTGGTTTTGA	2-10-2	cEt	60	345
481515	4325	4340	AGAACTCTGATCAGC	3-10-3	cEt	88	346
481740	4326	4339	GAACTCTGATCAG	2-10-2	cEt	71	347
481516	4364	4379	CAGAGACCAGCTAATT	3-10-3	cEt	78	348
481741	4365	4378	AGAGACCAGCTAAT	2-10-2	cEt	80	349
481517	4394	4409	ATCTTAGAGAAGGTCG	3-10-3	cEt	87	350
481742	4395	4408	TCTTAGAGAAGGTC	2-10-2	cEt	64	351
481518	4425	4440	CCAGGCAGGAGGACTG	3-10-3	cEt	67	352

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481743	4426	4439	CAGGCAGGAGGACT	2-10-2	cEt	75	353
481519	4437	4452	CATCAACTGTCTCCAG	3-10-3	cEt	29	354
481744	4438	4451	ATCAACTGTCTCCA	2-10-2	cEt	69	355
481520	4439	4454	CACATCAACTGTCTCC	3-10-3	cEt	73	356
481745	4440	4453	ACATCAACTGTCTC	2-10-2	cEt	74	357
481521	4459	4474	GAAGTAAGAGCTCTGC	3-10-3	cEt	86	358
481746	4460	4473	AAGTAAGAGCTCTG	2-10-2	cEt	67	359
481522	4474	4489	AAGAGTGTTGCTGGAG	3-10-3	cEt	92	360
481747	4475	4488	AGAGTGTTGCTGGA	2-10-2	cEt	95	361
481523	4489	4504	GCTTATTATGTACTGA	3-10-3	cEt	95	362
481748	4490	4503	CTTATTATGTACTG	2-10-2	cEt	15	363
481524	4530	4545	GCCCAAGTCTCACCTT	3-10-3	cEt	70	364
481749	4531	4544	CCCAAGTCTCACCT	2-10-2	cEt	70	365
481525	4541	4556	CCCAATGGTAAGCCCA	3-10-3	cEt	93	366
481750	4542	4555	CCAATGGTAAGCCC	2-10-2	cEt	94	367
481526	4543	4558	AACCCAATGGTAAGCC	3-10-3	cEt	82	368
481751	4544	4557	ACCCAATGGTAAGC	2-10-2	cEt	54	369
481527	4560	4575	TAGGTCCCTATGATTT	3-10-3	cEt	55	370
481752	4561	4574	AGGTCCCTATGATT	2-10-2	cEt	62	371
481528	4579	4594	AAGCCCTGAACCCTCG	3-10-3	cEt	77	372
481753	4580	4593	AGCCCTGAACCCTC	2-10-2	cEt	71	373
481529	4615	4630	CCTAAGGCCATGAAC	3-10-3	cEt	64	374
481754	4616	4629	CTAAGGCCATGAAC	2-10-2	cEt	53	375
481530	4630	4645	ACCAGATACATGCTAC	3-10-3	cEt	87	376
481755	4631	4644	CCAGATACATGCTA	2-10-2	cEt	84	377
481531	4646	4661	TACAATCAGAGTTAAG	3-10-3	cEt	66	378
481756	4647	4660	ACAATCAGAGTTAA	2-10-2	cEt	5	379
481532	4664	4679	TCCTCTCAGAACTTTT	3-10-3	cEt	65	380
481757	4665	4678	CCTCTCAGAACTTT	2-10-2	cEt	81	381
481533	4666	4681	GCTCCTCTCAGAACTT	3-10-3	cEt	80	382
481758	4667	4680	CTCCTCTCAGAACT	2-10-2	cEt	62	383
481534	4693	4708	TTCTTTAATGGGCCAC	3-10-3	cEt	79	384
481759	4694	4707	TCTTTAATGGGCCA	2-10-2	cEt	74	385
481535	4767	4782	ACGGGATTCCCTCGGC	3-10-3	cEt	78	386
481760	4768	4781	CGGGATTCCCTCGG	2-10-2	cEt	78	387
481536	4782	4797	GTAGGTAAGCAACCCA	3-10-3	cEt	91	388
481761	4783	4796	TAGGTAAGCAACCC	2-10-2	cEt	78	389
481537	4830	4845	GAATTTGAATGCAGTG	3-10-3	cEt	84	390
481762	4831	4844	AATTTGAATGCAGT	2-10-2	cEt	2	391
481538	4844	4859	TGAAGTACACATTGGA	3-10-3	cEt	92	392
481763	4845	4858	GAAGTACACATTGG	2-10-2	cEt	96	393
481539	4860	4875	ATAAATTTTTACTACTA	3-10-3	cEt	19	394

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481764	4861	4874	TAAATTTTACACT	2-10-2	cEt	1	395
481765	4869	4882	CAATAATATAAATT	2-10-2	cEt	0	396
481541	4934	4949	CTGGAAGTTAAAGTAG	3-10-3	cEt	71	397
481766	4935	4948	TGGAAGTTAAAGTA	2-10-2	cEt	10	398

Table 2

Inhibition of human STAT3 mRNA levels by cEt and MOE chimeric antisense oligonucleotides targeted to
SEQ ID NO: 2

ISIS NO	Human Start Site	Human Stop Site	Sequence	Motif	Wing Chem	% inhibition	SEQ ID NO
481350	1065	1080	TCCAGGATCCGGTTGG	3-10-3	cEt	52	9
481575	1066	1079	CCAGGATCCGGTTG	2-10-2	cEt	41	10
481351	1121	1136	GGCCGAAGGGCCTCTC	3-10-3	cEt	14	11
481576	1122	1135	GCCGAAGGGCCTCT	2-10-2	cEt	8	12
481542	1988	2003	GGCTCAATTATTTATC	3-10-3	cEt	64	399
481767	1989	2002	GCTCAATTATTTAT	2-10-2	cEt	0	400
481543	1996	2011	AATGCAATGGCTCAAT	3-10-3	cEt	84	401
481768	1997	2010	ATGCAATGGCTCAA	2-10-2	cEt	95	402
481544	2004	2019	ATCCAGTAAATGCAAT	3-10-3	cEt	58	403
481769	2005	2018	TCCAGTAAATGCAA	2-10-2	cEt	55	404
481545	2061	2076	AGAAAACTCCCCTCT	3-10-3	cEt	36	405
481770	2062	2075	GAAAACTCCCCTCT	2-10-2	cEt	42	406
481546	2113	2128	CTGTCTTTGTTTCCCT	3-10-3	cEt	70	407
481771	2114	2127	TGTCTTTGTTTCCC	2-10-2	cEt	75	408
481547	2121	2136	AGGCCAGCCTGTCTTT	3-10-3	cEt	87	409
481772	2122	2135	GGCCAGCCTGTCTT	2-10-2	cEt	53	410
481548	2705	2720	CTAATGGTTCTTTGTG	3-10-3	cEt	78	411
481773	2706	2719	TAATGGTTCTTTGT	2-10-2	cEt	9	412
481549	6476	6491	GAAATTCATTCTTCCA	3-10-3	cEt	96	413
481774	6477	6490	AAATTCATTCTTCC	2-10-2	cEt	56	414
481550	10001	10016	ACACACACAGATGTGA	3-10-3	cEt	48	415
481775	10002	10015	CACACACAGATGTG	2-10-2	cEt	35	416
481551	10337	10352	CTACCCAAACATCCCC	3-10-3	cEt	69	417
481776	10338	10351	TACCCAAACATCCC	2-10-2	cEt	62	418
481552	10345	10360	TACAAAACTACCCAA	3-10-3	cEt	30	419
481777	10346	10359	ACAAAACTACCCA	2-10-2	cEt	1	420
481553	10364	10379	AGTTTTCAGAAATGGC	3-10-3	cEt	96	421
481778	10365	10378	GTTTTCAGAAATGG	2-10-2	cEt	47	422
481554	15469	15484	CAAGCTTTTCTATGAA	3-10-3	cEt	86	423
481779	15470	15483	AAGCTTTTCTATGA	2-10-2	cEt	60	424
481555	24588	24603	TTATTCAGGTCACCTT	3-10-3	cEt	73	425
481780	24589	24602	TATTCAGGTCACCT	2-10-2	cEt	60	426

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481352	40953	40968	CCTGCTAAAATCAGGG	3-10-3	cEt	15	13
481577	40954	40967	CTGCTAAAATCAGG	2-10-2	cEt	12	14
481353	40968	40983	ATTCCATTGGGCCATC	3-10-3	cEt	78	15
481578	40969	40982	TTCCATTGGGCCAT	2-10-2	cEt	51	16
481354	40992	41007	CCGTGTGTCAAGCTGC	3-10-3	cEt	98	17
481579	40993	41006	CGTGTGTCAAGCTG	2-10-2	cEt	91	18
481355	41050	41065	ACTGCCGCAGCTCCAT	3-10-3	cEt	95	19
481580	41051	41064	CTGCCGCAGCTCCA	2-10-2	cEt	76	20
481356	41074	41089	GACTCTCAATCCAAGG	3-10-3	cEt	83	21
481581	41075	41088	ACTCTCAATCCAAG	2-10-2	cEt	31	22
481556	42765	42780	GCATATGCCCTAGGAA	3-10-3	cEt	23	430
481781	42766	42779	CATATGCCCTAGGA	2-10-2	cEt	15	431
481357	42778	42793	TTCTTTGCTGGCCGCA	3-10-3	cEt	97	23
481582	42779	42792	TCTTTGCTGGCCGC	2-10-2	cEt	87	24
481358	42806	42821	GATTATGAAACACCAA	3-10-3	cEt	85	25
481583	42807	42820	ATTATGAAACACCA	2-10-2	cEt	20	26
481359	42832	42847	ATACTGCTGGTCAATC	3-10-3	cEt	90	27
481584	42833	42846	TACTGCTGGTCAAT	2-10-2	cEt	42	28
481360	42862	42877	GAGAACATTCGACTCT	3-10-3	cEt	75	29
481585	42863	42876	AGAACATTCGACTC	2-10-2	cEt	77	30
481361	42877	42892	TAGATTGTGCTGATAG	3-10-3	cEt	90	31
481586	42878	42891	AGATTGTGCTGATA	2-10-2	cEt	81	32
481362	42893	42908	ACTGCTTGATTCTTCG	3-10-3	cEt	59	33
481587	42894	42907	CTGCTTGATTCTTC	2-10-2	cEt	23	34
481557	43043	43058	GCTAATTACTTCTCCT	3-10-3	cEt	57	432
481782	43044	43057	CTAATTACTTCTCC	2-10-2	cEt	25	433
481588	43826	43839	AAGATACCTGCTCT	2-10-2	cEt	58	36
481364	43856	43871	GCCACAATCCGGGCAA	3-10-3	cEt	36	37
481589	43857	43870	CCACAATCCGGGCA	2-10-2	cEt	69	38
481365	43903	43918	CAGTGGCTGCAGTCTG	3-10-3	cEt	36	39
481590	43904	43917	AGTGGCTGCAGTCT	2-10-2	cEt	30	40
481558	50069	50084	GCCCCCTTGCTGCCAA	3-10-3	cEt	0	434
481783	50070	50083	CCCCCTTGCTGCCA	2-10-2	cEt	39	435
481367	50101	50116	GTCACCACGGCTGCTG	3-10-3	cEt	70	43
481592	50102	50115	TCACCACGGCTGCT	2-10-2	cEt	48	44
481368	50122	50137	TCCAGCATCTGCTGCT	3-10-3	cEt	81	45
481593	50123	50136	CCAGCATCTGCTGC	2-10-2	cEt	46	46
481369	50138	50153	ATCCTGAAGGTGCTGC	3-10-3	cEt	29	47
481594	50139	50152	TCCTGAAGGTGCTG	2-10-2	cEt	16	48
481559	50668	50683	TGTTCTAGATCCTGTT	3-10-3	cEt	72	436
481784	50669	50682	GTTCTAGATCCTGT	2-10-2	cEt	79	437
481371	50673	50688	TTTTCTGTTCTAGATC	3-10-3	cEt	83	51

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481596	50674	50687	TTTCTGTTCTAGAT	2-10-2	cEt	48	52
481372	50694	50709	GGAGATTCTCTACCAC	3-10-3	cEt	85	53
481597	50695	50708	GAGATTCTCTACCA	2-10-2	cEt	80	54
481373	50715	50730	AGTTGAAATCAAAGTC	3-10-3	cEt	87	55
481598	50716	50729	GTTGAAATCAAAGT	2-10-2	cEt	6	56
481599	51626	51639	GATCTTGCATGTCT	2-10-2	cEt	51	58
481375	51636	51651	TGTTTCCATTTCAGATC	3-10-3	cEt	65	59
481600	51637	51650	GTTTCCATTTCAGAT	2-10-2	cEt	42	60
481376	51705	51720	TCCGCATCTGGTCCAG	3-10-3	cEt	82	61
481601	51706	51719	CCGCATCTGGTCCA	2-10-2	cEt	70	62
481560	51708	51723	CTCTCCGCATCTGGTC	3-10-3	cEt	63	438
481785	51709	51722	TCTCCGCATCTGGT	2-10-2	cEt	28	63
481378	51905	51920	CCGCCAGCTCACTCAC	3-10-3	cEt	89	66
481603	51906	51919	CGCCAGCTCACTCA	2-10-2	cEt	60	67
481379	51968	51983	TCCAGTCAGCCAGCTC	3-10-3	cEt	91	68
481604	51969	51982	CCAGTCAGCCAGCT	2-10-2	cEt	70	69
481380	51976	51991	CCGCCTCTTCCAGTCA	3-10-3	cEt	73	70
481605	51977	51990	CGCCTCTTCCAGTC	2-10-2	cEt	55	71
481381	52023	52038	CGATCTAGGCAGATGT	3-10-3	cEt	26	72
481606	52024	52037	GATCTAGGCAGATG	2-10-2	cEt	35	73
481382	55443	55458	GAGATTCTGCTAATGA	3-10-3	cEt	81	74
481607	55444	55457	AGATTCTGCTAATG	2-10-2	cEt	51	75
481383	55451	55466	CTGAAGTTGAGATTCT	3-10-3	cEt	84	76
481608	55452	55465	TGAAGTTGAGATTC	2-10-2	cEt	26	77
481384	55496	55511	AACTTTTGTGCAAC	3-10-3	cEt	76	78
481609	55497	55510	ACTTTTGTGCAA	2-10-2	cEt	34	79
481385	55511	55526	GTCCCTTTGTAGGAA	3-10-3	cEt	41	80
481610	55512	55525	TCCCTTTGTAGGA	2-10-2	cEt	37	81
481387	55748	55763	CAGGATGCATGGGCAT	3-10-3	cEt	92	84
481612	55749	55762	AGGATGCATGGGCA	2-10-2	cEt	86	85
481388	55792	55807	TTAGTAGTGAAGTGG	3-10-3	cEt	74	86
481613	55793	55806	TTAGTAGTGAAGT	2-10-2	cEt	22	87
481561	57949	57964	TGACCAGCAACCTATT	3-10-3	cEt	43	439
481786	57950	57963	GACCAGCAACCTAT	2-10-2	cEt	59	440
481390	57969	57984	ATAATTCAACTCAGGG	3-10-3	cEt	92	90
481615	57970	57983	TAATTCAACTCAGG	2-10-2	cEt	48	91
481391	57978	57993	TTAAGCTGATAATTC	3-10-3	cEt	44	92
481616	57979	57992	TTAAGCTGATAATT	2-10-2	cEt	0	93
481392	57990	58005	GCACACTTTAATTTTA	3-10-3	cEt	49	94
481617	57991	58004	CACACTTTAATTTT	2-10-2	cEt	1	95
481562	59703	59718	CCCAGAGTCTCTGTAA	3-10-3	cEt	36	441
481787	59704	59717	CCAGAGTCTCTGTA	2-10-2	cEt	22	442

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481394	59895	59910	GAGGCTGCCGTTGTTG	3-10-3	cEt	62	98
481619	59896	59909	AGGCTGCCGTTGTT	2-10-2	cEt	29	99
481396	60034	60049	CACATCTCTGCTCCCT	3-10-3	cEt	92	102
481621	60035	60048	ACATCTCTGCTCCC	2-10-2	cEt	74	103
481563	60064	60079	TTACATCACAATTGGC	3-10-3	cEt	24	445
481788	60065	60078	TACATCACAATTGG	2-10-2	cEt	3	446
481398	63306	63321	GATCAGGTGCAGCTCC	3-10-3	cEt	73	106
481623	63307	63320	ATCAGGTGCAGCTC	2-10-2	cEt	40	107
481399	63327	63342	ATACACCTCGGTCTCA	3-10-3	cEt	73	108
481624	63328	63341	TACACCTCGGTCTC	2-10-2	cEt	43	109
481400	63343	63358	TCTTGAGGCCTTGGTG	3-10-3	cEt	47	110
481625	63344	63357	CTTGAGGCCTTGGT	2-10-2	cEt	16	111
481401	63353	63368	TCTAGGTCAATCTTGA	3-10-3	cEt	74	112
481626	63354	63367	CTAGGTCAATCTTG	2-10-2	cEt	54	113
481564	64421	64436	GCAAGGAGTGGGTCTG	3-10-3	cEt	33	446
481789	64422	64435	CAAGGAGTGGGTCT	2-10-2	cEt	10	116
481403	64425	64440	ACTGGCAAGGAGTGGG	3-10-3	cEt	58	117
481628	64426	64439	CTGGCAAGGAGTGG	2-10-2	cEt	38	118
481404	64451	64466	TCTGACAGATGTTGGA	3-10-3	cEt	50	119
481629	64452	64465	CTGACAGATGTTGG	2-10-2	cEt	64	120
481405	64459	64474	ATTTGGCATCTGACAG	3-10-3	cEt	75	121
481630	64460	64473	TTTGGCATCTGACA	2-10-2	cEt	39	122
481407	64663	64678	CCCAGGTTCCAATTGG	3-10-3	cEt	50	125
481632	64664	64677	CCAGGTTCCAATTG	2-10-2	cEt	32	126
481408	64714	64729	CTCGCTTGGTGGTGG	3-10-3	cEt	53	127
481633	64715	64728	TCGCTTGGTGGTGG	2-10-2	cEt	35	128
481409	64729	64744	GCTCGATGCTCAGTCC	3-10-3	cEt	86	129
481634	64730	64743	CTCGATGCTCAGTC	2-10-2	cEt	43	130
481410	64759	64774	CCAAGAGTTTCTCTGC	3-10-3	cEt	91	131
481635	64760	64773	CAAGAGTTTCTCTG	2-10-2	cEt	43	132
481411	65859	65874	AATTCACACCAGGTCC	3-10-3	cEt	72	133
481636	65860	65873	ATTCACACCAGGTC	2-10-2	cEt	42	134
481412	65877	65892	TGATCTGACACCCTGA	3-10-3	cEt	90	135
481637	65878	65891	GATCTGACACCCTG	2-10-2	cEt	79	136
481413	65885	65900	AGCCCATGTGATCTGA	3-10-3	cEt	80	137
481638	65886	65899	GCCCATGTGATCTG	2-10-2	cEt	64	138
481565	66119	66134	TTTCCTGGAGAAAAGA	3-10-3	cEt	4	447
481790	66120	66133	TTCCTGGAGAAAAG	2-10-2	cEt	3	448
481566	66127	66142	AGCCATGTTTTCCTGG	3-10-3	cEt	62	449
481791	66128	66141	GCCATGTTTTCCTG	2-10-2	cEt	73	450
481415	66133	66148	CTTGCCAGCCATGTTT	3-10-3	cEt	88	141
481640	66134	66147	TTGCCAGCCATGTT	2-10-2	cEt	57	142

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337332	66135	66154	GAAGCCCTTGCCAGCCATGT	5-10-5	MOE	63	143
481416	66138	66153	AAGCCCTTGCCAGCCA	3-10-3	cEt	87	144
481641	66139	66152	AGCCCTTGCCAGCC	2-10-2	cEt	68	145
337333	66140	66159	AAGGAGAAGCCCTTGCCAGC	5-10-5	MOE	49	146
481417	66140	66155	AGAAGCCCTTGCCAGC	3-10-3	cEt	97	147
481418	66141	66156	GAGAAGCCCTTGCCAG	3-10-3	cEt	92	148
481642	66141	66154	GAAGCCCTTGCCAG	2-10-2	cEt	67	149
481419	66142	66157	GGAGAAGCCCTTGCCA	3-10-3	cEt	83	150
481643	66142	66155	AGAAGCCCTTGCCA	2-10-2	cEt	58	151
481644	66143	66156	GAGAAGCCCTTGCC	2-10-2	cEt	45	152
481420	66185	66200	ACTTTTTCACAAGGTC	3-10-3	cEt	94	153
481645	66186	66199	CTTTTTCACAAGGT	2-10-2	cEt	50	154
481421	66374	66389	CTCAAGATGGCCCGCT	3-10-3	cEt	86	155
481646	66375	66388	TCAAGATGGCCCGC	2-10-2	cEt	41	156
481422	66389	66404	CCTGGAGGCTTAGTGC	3-10-3	cEt	80	157
481647	66390	66403	CTGGAGGCTTAGTG	2-10-2	cEt	0	158
481423	66430	66445	CTCCTTCTTTGCTGCT	3-10-3	cEt	69	159
481648	66431	66444	TCCTTCTTTGCTGC	2-10-2	cEt	51	160
481424	66446	66461	CAAGTGAAAGTGACGC	3-10-3	cEt	70	161
481649	66447	66460	AAGTGAAAGTGACG	2-10-2	cEt	25	162
481425	66468	66483	ACCGCTGATGTCCTTC	3-10-3	cEt	78	163
481650	66469	66482	CCGCTGATGTCCTT	2-10-2	cEt	79	164
481426	66993	67008	ACTGGATCTGGGTCTT	3-10-3	cEt	80	165
481651	66994	67007	CTGGATCTGGGTCT	2-10-2	cEt	64	166
481427	67017	67032	GCTGCTTTGTGTATGG	3-10-3	cEt	75	167
481652	67018	67031	CTGCTTTGTGTATG	2-10-2	cEt	82	168
481428	67026	67041	TGTTTCAGCTGCTGCTT	3-10-3	cEt	77	169
481653	67027	67040	GTTCAGCTGCTGCT	2-10-2	cEt	79	170
481429	67034	67049	TGACATGTTGTTTCAGC	3-10-3	cEt	84	171
481654	67035	67048	GACATGTTGTTTCAG	2-10-2	cEt	70	172
481430	67052	67067	CATGATGATTTTCAGCA	3-10-3	cEt	67	173
481655	67053	67066	ATGATGATTTTCAGC	2-10-2	cEt	31	174
481431	67068	67083	CCATGATCTTATAGCC	3-10-3	cEt	91	175
481656	67069	67082	CATGATCTTATAGC	2-10-2	cEt	0	176
481432	67095	67110	GTGGAGACACCAGGAT	3-10-3	cEt	55	177
481657	67096	67109	TGGAGACACCAGGA	2-10-2	cEt	58	178
481433	67118	67133	AATGTCAGGATAGAGA	3-10-3	cEt	73	179
481658	67119	67132	ATGTCAGGATAGAG	2-10-2	cEt	62	180
481434	67128	67143	CCTCCTTGGAATGTC	3-10-3	cEt	73	181
345785	67129	67148	TGCCTCCTCCTTGGAATGT	5-10-5	MOE	50	182
481659	67129	67142	CTCCTTGGAATGT	2-10-2	cEt	51	183
481435	67131	67146	CCTCCTCCTTGGAAT	3-10-3	cEt	49	184

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481660	67132	67145	CTCCTCCTTGGGAA	2-10-2	cEt	54	185
481436	67137	67152	CGAATGCCTCCTCCTT	3-10-3	cEt	82	186
481661	67138	67151	GAATGCCTCCTCCT	2-10-2	cEt	76	187
481437	67158	67173	TCTCTGGCCGACAATA	3-10-3	cEt	49	188
481662	67159	67172	CTCTGGCCGACAAT	2-10-2	cEt	43	189
481567	67194	67209	AACAACCTACCTGGGTC	3-10-3	cEt	20	451
481792	67195	67208	ACAACCTACCTGGGT	2-10-2	cEt	0	452
481438	72272	72287	ACTTGGTCTTCAGGTA	3-10-3	cEt	51	190
481663	72273	72286	CTTGGTCTTCAGGT	2-10-2	cEt	52	191
481568	72290	72305	ACGGTGTACACAGAT	3-10-3	cEt	85	453
481793	72291	72304	CGGTGTACACAGA	2-10-2	cEt	93	454
481569	72430	72445	AACACACAAGGTCACT	3-10-3	cEt	62	455
481794	72431	72444	ACACACAAGGTCAC	2-10-2	cEt	81	456
481570	72438	72453	GCTTTTTAAACACACA	3-10-3	cEt	79	457
481795	72439	72452	CTTTTTAAACACAC	2-10-2	cEt	0	458
481571	72528	72543	TGACAAGACACAATGG	3-10-3	cEt	12	459
481796	72529	72542	GACAAGACACAATG	2-10-2	cEt	36	460
481440	72586	72601	GTATTGCTGCAGGTCG	3-10-3	cEt	79	194
481665	72587	72600	TATTGCTGCAGGTC	2-10-2	cEt	43	195
481441	72594	72609	GGTCAATGGTATTGCT	3-10-3	cEt	55	196
481666	72595	72608	GTCAATGGTATTGC	2-10-2	cEt	36	197
481442	72602	72617	CATCGGCAGGTCAATG	3-10-3	cEt	44	198
481667	72603	72616	ATCGGCAGGTCAAT	2-10-2	cEt	31	199
481443	72622	72637	GAATCTAAAGTGCGGG	3-10-3	cEt	78	200
481668	72623	72636	AATCTAAAGTGCGG	2-10-2	cEt	41	201
481444	72630	72645	GCATCAATGAATCTAA	3-10-3	cEt	66	202
481669	72631	72644	CATCAATGAATCTA	2-10-2	cEt	0	203
481445	72638	72653	TCCAAACTGCATCAAT	3-10-3	cEt	70	204
481670	72639	72652	CCAAACTGCATCAA	2-10-2	cEt	60	205
481446	72659	72674	TTCAGCACCTTCACCA	3-10-3	cEt	44	206
481671	72660	72673	TCAGCACCTTCACC	2-10-2	cEt	41	207
481447	72675	72690	GCCCTCCTGCTGAGGG	3-10-3	cEt	10	208
481672	72676	72689	CCCTCCTGCTGAGG	2-10-2	cEt	15	209
481572	72682	72697	CCAAACTGCCCTCCTG	3-10-3	cEt	51	461
481797	72683	72696	CAAACCTGCCCTCCT	2-10-2	cEt	11	211
481573	73535	73550	GGTCAGAAAAGCCAGA	3-10-3	cEt	55	462
481798	73536	73549	GTCAGAAAAGCCAG	2-10-2	cEt	59	463
481449	73690	73705	CCATGTCAAAGGTGAG	3-10-3	cEt	77	213
481674	73691	73704	CATGTCAAAGGTGA	2-10-2	cEt	31	214
481450	73717	73732	GGGAGGTAGCGCACTC	3-10-3	cEt	53	215
481675	73718	73731	GGAGGTAGCGCACT	2-10-2	cEt	41	216
481451	73779	73794	GAATGCAGGTAGGCGC	3-10-3	cEt	55	217

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481676	73780	73793	AATGCAGGTAGGCG	2-10-2	cEt	39	218
481452	73818	73833	TTTCAGATGATCTGGG	3-10-3	cEt	71	219
481677	73819	73832	TTCAGATGATCTGG	2-10-2	cEt	38	220
481574	73837	73852	GGAACCACAAAGTTAG	3-10-3	cEt	69	221
481799	73838	73851	GAACCACAAAGTTA	2-10-2	cEt	50	222
481453	73868	73883	GATAGCAGAAGTAGGA	3-10-3	cEt	92	223
481678	73869	73882	ATAGCAGAAGTAGG	2-10-2	cEt	78	224
481454	73889	73904	AAAGTGCCCAGATTGC	3-10-3	cEt	85	225
481679	73890	73903	AAGTGCCCAGATTG	2-10-2	cEt	69	226
481455	73909	73924	CACTCATTTCTCTATT	3-10-3	cEt	74	227
481680	73910	73923	ACTCATTTCTCTAT	2-10-2	cEt	39	228
481456	73954	73969	AACACATCCTTATTTG	3-10-3	cEt	48	229
481681	73955	73968	ACACATCCTTATTT	2-10-2	cEt	47	230
481457	73966	73981	TGGGTCTCAGAGAACA	3-10-3	cEt	88	231
481682	73967	73980	GGGTCTCAGAGAAC	2-10-2	cEt	77	232
481458	74019	74034	CAAGACATTTCCTTTT	3-10-3	cEt	54	233
481683	74020	74033	AAGACATTTCCTTT	2-10-2	cEt	29	234
481459	74095	74110	GGAGGCACTTGTCTAA	3-10-3	cEt	76	235
481684	74096	74109	GAGGCACTTGTCTA	2-10-2	cEt	89	236
481460	74130	74145	TTACAGAAACAGGCAG	3-10-3	cEt	83	237
481685	74131	74144	TACAGAAACAGGCA	2-10-2	cEt	36	238
481461	74156	74171	AGCTATAGGTGGCCTG	3-10-3	cEt	75	239
481686	74157	74170	GCTATAGGTGGCCT	2-10-2	cEt	70	240
481462	74171	74186	ATGCCAGGAGTATGTA	3-10-3	cEt	89	241
481687	74172	74185	TGCCAGGAGTATGT	2-10-2	cEt	80	242
481463	74188	74203	CAAGGTAAAAAGTGC	3-10-3	cEt	88	243
481688	74189	74202	AAGGTAAAAAGTG	2-10-2	cEt	13	244
481464	74203	74218	CTATTTGGATGTCAGC	3-10-3	cEt	97	245
481689	74204	74217	TATTTGGATGTCAG	2-10-2	cEt	40	246
481465	74219	74234	TAGATAGTCCTATCTT	3-10-3	cEt	51	247
481690	74220	74233	AGATAGTCCTATCT	2-10-2	cEt	64	248
481466	74234	74249	AAGAAACCTAGGGCTT	3-10-3	cEt	74	249
481691	74235	74248	AGAAACCTAGGGCT	2-10-2	cEt	77	250
481467	74284	74299	GCTGATACAGTGTTTT	3-10-3	cEt	74	251
481692	74285	74298	CTGATACAGTGTTT	2-10-2	cEt	74	252
481468	74299	74314	ATACAGAAAGGCTATG	3-10-3	cEt	71	253
481693	74300	74313	TACAGAAAGGCTAT	2-10-2	cEt	25	254
481469	74314	74329	GCTTAAGTTTCTTAAA	3-10-3	cEt	61	255
481694	74315	74328	CTTAAGTTTCTTAA	2-10-2	cEt	0	256
481470	74648	74663	AGCACCAAGGAGGCTG	3-10-3	cEt	49	257
481695	74649	74662	GCACCAAGGAGGCT	2-10-2	cEt	83	258
481471	74663	74678	AAGCTGAATGCTTAAA	3-10-3	cEt	36	259

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481696	74664	74677	AGCTGAATGCTTAA	2-10-2	cEt	33	260
481472	74678	74693	TTACCAGCCTGAAGGA	3-10-3	cEt	76	261
481697	74679	74692	TACCAGCCTGAAGG	2-10-2	cEt	63	262
481473	74693	74708	CAGGGATTATATAAAT	3-10-3	cEt	53	263
481698	74694	74707	AGGGATTATATAAA	2-10-2	cEt	15	264
481474	74708	74723	ACCTGAAGCCCGTTTC	3-10-3	cEt	80	265
481699	74709	74722	CCTGAAGCCCGTTT	2-10-2	cEt	57	266
481475	74723	74738	TGTCTTAAGGGTTTGA	3-10-3	cEt	93	267
481700	74724	74737	GTCTTAAGGGTTTG	2-10-2	cEt	89	268
481476	74738	74753	GGTTGCAGCTTCAGAT	3-10-3	cEt	92	269
481701	74739	74752	GTTGCAGCTTCAGA	2-10-2	cEt	60	270
481477	74754	74769	TCAACACCAAAGGCCA	3-10-3	cEt	95	271
481702	74755	74768	CAACACCAAAGGCC	2-10-2	cEt	89	272
481478	74772	74787	TCCTTAAACCTTCCTA	3-10-3	cEt	84	273
481703	74773	74786	CCTTAAACCTTCCT	2-10-2	cEt	57	274
481479	74787	74802	AAAATGCTTAGATTCT	3-10-3	cEt	80	275
481704	74788	74801	AAATGCTTAGATTC	2-10-2	cEt	32	276
481480	74815	74830	AAATAAGTCTATTTAT	3-10-3	cEt	5	277
481705	74816	74829	AATAAGTCTATTTA	2-10-2	cEt	25	278
481481	74835	74850	GGCCAATACATTACAA	3-10-3	cEt	63	279
481706	74836	74849	GCCAATACATTACA	2-10-2	cEt	56	280
481482	74857	74872	TGCCCAGCCTTACTCA	3-10-3	cEt	55	281
481707	74858	74871	GCCCAGCCTTACTC	2-10-2	cEt	43	282
481483	74872	74887	GTTGTAAGCACCTCT	3-10-3	cEt	1	283
481708	74873	74886	TTGTAAGCACCTC	2-10-2	cEt	56	284
481484	74887	74902	AGAAAGGGAGTCAAGG	3-10-3	cEt	60	285
481709	74888	74901	GAAAGGGAGTCAAG	2-10-2	cEt	27	286
481485	74904	74919	GCAGATCAAGTCCAGG	3-10-3	cEt	90	287
481710	74905	74918	CAGATCAAGTCCAG	2-10-2	cEt	88	288
481486	74917	74932	AGCCTCTGAAACAGCA	3-10-3	cEt	75	289
481711	74918	74931	GCCTCTGAAACAGC	2-10-2	cEt	74	290
481487	74933	74948	CCCACAGAAACAACCT	3-10-3	cEt	66	291
481712	74934	74947	CCACAGAAACAACC	2-10-2	cEt	45	292
481488	74948	74963	AGCCCTGATAAGGCAC	3-10-3	cEt	23	293
481713	74949	74962	GCCCTGATAAGGCA	2-10-2	cEt	18	294
481489	74963	74978	AATCAGAAGTATCCCA	3-10-3	cEt	60	295
481714	74964	74977	ATCAGAAGTATCCC	2-10-2	cEt	43	296
481490	75020	75035	GCCTCTAGCAGGATCA	3-10-3	cEt	78	297
481715	75021	75034	CCTCTAGCAGGATC	2-10-2	cEt	79	298
481491	75035	75050	CACGCAAGGAGACATG	3-10-3	cEt	70	299
481716	75036	75049	ACGCAAGGAGACAT	2-10-2	cEt	68	300
481492	75050	75065	TGAGGGACCTTTAGAC	3-10-3	cEt	61	301

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481717	75051	75064	GAGGGACCTTTAGA	2-10-2	cEt	44	302
481493	75073	75088	CAGGATTCCTAAAACA	3-10-3	cEt	43	303
481718	75074	75087	AGGATTCCTAAAAC	2-10-2	cEt	7	304
481494	75088	75103	ATGAGGTCCTGAGACC	3-10-3	cEt	60	305
481719	75089	75102	TGAGGTCCTGAGAC	2-10-2	cEt	29	306
481495	75127	75142	CATCATGTCCAACCTG	3-10-3	cEt	92	307
481720	75128	75141	ATCATGTCCAACCT	2-10-2	cEt	63	308
481496	75142	75157	GGGCCCCATAGTGTGC	3-10-3	cEt	29	309
481721	75143	75156	GGCCCCATAGTGTG	2-10-2	cEt	19	310
481497	75164	75179	AGCTCAACCAGACACG	3-10-3	cEt	67	311
481722	75165	75178	GCTCAACCAGACAC	2-10-2	cEt	69	312
481498	75179	75194	GAACCATATTCCTGA	3-10-3	cEt	90	313
481723	75180	75193	AACCATATTCCTG	2-10-2	cEt	49	314
481499	75194	75209	CAAGAACTGGCTAAG	3-10-3	cEt	43	315
481724	75195	75208	AAGAACTGGCTAA	2-10-2	cEt	17	316
481500	75209	75224	GCCACTGGATATCACC	3-10-3	cEt	92	317
481725	75210	75223	CCACTGGATATCAC	2-10-2	cEt	88	464
481501	75235	75250	AACTGAATGAAGACGC	3-10-3	cEt	91	318
481726	75236	75249	ACTGAATGAAGACG	2-10-2	cEt	56	319
481502	75250	75265	CCTTTGCCCTGCATGA	3-10-3	cEt	85	320
481727	75251	75264	CTTTGCCCTGCATG	2-10-2	cEt	70	321
481503	75265	75280	AAGTTTATCAGTAAGC	3-10-3	cEt	57	322
481728	75266	75279	AGTTTATCAGTAAG	2-10-2	cEt	22	323
481504	75280	75295	TACGAGGGCAGACTCA	3-10-3	cEt	60	324
481729	75281	75294	ACGAGGGCAGACTC	2-10-2	cEt	22	325
481505	75295	75310	AGGTATACACCCTCAT	3-10-3	cEt	45	326
481730	75296	75309	GGTATACACCCTCA	2-10-2	cEt	47	327
481506	75310	75325	CCTCAGAGGGAGGCCA	3-10-3	cEt	32	328
481731	75311	75324	CTCAGAGGGAGGCC	2-10-2	cEt	0	329
481507	75325	75340	GGGAGGAGTCACCAGC	3-10-3	cEt	64	330
481732	75326	75339	GGAGGAGTCACCAG	2-10-2	cEt	59	331
481508	75392	75407	TAGCCAGCCAAGGCGG	3-10-3	cEt	33	332
481733	75393	75406	AGCCAGCCAAGGCG	2-10-2	cEt	50	333
481509	75407	75422	ACAGGAGAGGCGAGCT	3-10-3	cEt	46	334
481734	75408	75421	CAGGAGAGGCGAGC	2-10-2	cEt	28	335
481510	75424	75439	TAGGTGTTCCCATACG	3-10-3	cEt	95	336
481735	75425	75438	AGGTGTTCCCATAC	2-10-2	cEt	22	337
481511	75445	75460	GGCAGCCCATCCAGCA	3-10-3	cEt	43	338
481736	75446	75459	GCAGCCCATCCAGC	2-10-2	cEt	54	339
481512	75462	75477	CATGCCTCTGAGTCAG	3-10-3	cEt	30	340
481737	75463	75476	ATGCCTCTGAGTCA	2-10-2	cEt	31	341
481513	75477	75492	GTTGCCAAATCCGGCC	3-10-3	cEt	85	342

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481738	75478	75491	TTGCCAAATCCGGC	2-10-2	cEt	70	343
481514	75492	75507	GCAAGGTGGTTTTGAG	3-10-3	cEt	85	344
481739	75493	75506	CAAGGTGGTTTTGA	2-10-2	cEt	60	345
481515	75512	75527	AGAAACTCTGATCAGC	3-10-3	cEt	88	346
481740	75513	75526	GAAACTCTGATCAG	2-10-2	cEt	71	347
481516	75551	75566	CAGAGACCAGCTAATT	3-10-3	cEt	78	348
481741	75552	75565	AGAGACCAGCTAAT	2-10-2	cEt	80	349
481517	75581	75596	ATCTTAGAGAAGGTCG	3-10-3	cEt	87	350
481742	75582	75595	TCTTAGAGAAGGTC	2-10-2	cEt	64	351
481518	75612	75627	CCAGGCAGGAGGACTG	3-10-3	cEt	67	352
481743	75613	75626	CAGGCAGGAGGACT	2-10-2	cEt	75	353
481519	75624	75639	CATCAACTGTCTCCAG	3-10-3	cEt	29	354
481744	75625	75638	ATCAACTGTCTCCA	2-10-2	cEt	69	355
481520	75626	75641	CACATCAACTGTCTCC	3-10-3	cEt	73	356
481745	75627	75640	ACATCAACTGTCTC	2-10-2	cEt	74	357
481521	75646	75661	GAAGTAAGAGCTCTGC	3-10-3	cEt	86	358
481746	75647	75660	AAGTAAGAGCTCTG	2-10-2	cEt	67	359
481522	75661	75676	AAGAGTGTTGCTGGAG	3-10-3	cEt	92	360
481747	75662	75675	AGAGTGTTGCTGGA	2-10-2	cEt	95	361
481523	75676	75691	GCTTATTATGTACTGA	3-10-3	cEt	95	362
481748	75677	75690	CTTATTATGTACTG	2-10-2	cEt	15	363
481524	75717	75732	GCCCAAGTCTCACCTT	3-10-3	cEt	70	364
481749	75718	75731	CCCAAGTCTCACCT	2-10-2	cEt	70	365
481525	75728	75743	CCCAATGGTAAGCCCA	3-10-3	cEt	93	366
481750	75729	75742	CCAATGGTAAGCCC	2-10-2	cEt	94	367
481526	75730	75745	AACCCAATGGTAAGCC	3-10-3	cEt	82	368
481751	75731	75744	ACCCAATGGTAAGC	2-10-2	cEt	54	369
481527	75747	75762	TAGGTCCCTATGATTT	3-10-3	cEt	55	370
481752	75748	75761	AGGTCCCTATGATT	2-10-2	cEt	62	371
481528	75766	75781	AAGCCCTGAACCCTCG	3-10-3	cEt	77	372
481753	75767	75780	AGCCCTGAACCCTC	2-10-2	cEt	71	373
481529	75802	75817	CCTAAGGCCATGAAC	3-10-3	cEt	64	374
481754	75803	75816	CTAAGGCCATGAAC	2-10-2	cEt	53	375
481530	75817	75832	ACCAGATACATGCTAC	3-10-3	cEt	87	376
481755	75818	75831	CCAGATACATGCTA	2-10-2	cEt	84	377
481531	75833	75848	TACAATCAGAGTTAAG	3-10-3	cEt	66	378
481756	75834	75847	ACAATCAGAGTTAA	2-10-2	cEt	5	379
481532	75851	75866	TCCTCTCAGAACTTTT	3-10-3	cEt	65	380
481757	75852	75865	CCTCTCAGAACTTT	2-10-2	cEt	81	381
481533	75853	75868	GCTCCTCTCAGAACTT	3-10-3	cEt	80	382
481758	75854	75867	CTCCTCTCAGAACT	2-10-2	cEt	62	383
481534	75880	75895	TTCTTTAATGGGCCAC	3-10-3	cEt	79	384

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481759	75881	75894	TCTTTAATGGGCCA	2-10-2	cEt	74	385
481535	75954	75969	ACGGGATTCCCTCGGC	3-10-3	cEt	78	386
481760	75955	75968	CGGGATTCCCTCGG	2-10-2	cEt	78	387
481536	75969	75984	GTAGGTAAGCAACCCA	3-10-3	cEt	91	388
481761	75970	75983	TAGGTAAGCAACCC	2-10-2	cEt	78	389
481537	76017	76032	GAATTTGAATGCAGTG	3-10-3	cEt	84	390
481762	76018	76031	AATTTGAATGCAGT	2-10-2	cEt	2	391
481538	76031	76046	TGAAGTACACATTGGA	3-10-3	cEt	92	392
481763	76032	76045	GAAGTACACATTGG	2-10-2	cEt	96	393
481539	76047	76062	ATAAATTTTACACTA	3-10-3	cEt	19	394
481764	76048	76061	TAAATTTTACACT	2-10-2	cEt	1	395
481765	76056	76069	CAATAATATAAATT	2-10-2	cEt	0	396
481541	76121	76136	CTGGAAGTTAAAGTAG	3-10-3	cEt	71	397
481766	76122	76135	TGGAAGTTAAAGTA	2-10-2	cEt	10	398

Example 2: Antisense inhibition of murine STAT3 in b.END cells

Antisense oligonucleotides tested in the study described in Example 1 were also tested for their effects on STAT3 mRNA in b.END cells. Cultured b.END cells at a density of 20,000 cells per well were transfected using electroporation with 7,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Murine primer probe set RTS2381 (forward sequence GCCACGTTGGTGTTCATAATCT, designated herein as SEQ ID NO: 465; reverse sequence GATAGAGGACATTGGACTCTTGCA, designated herein as SEQ ID NO: 466; probe sequence TTGGGTGAAATTGACCAGCAATATAGCCG, designated herein as SEQ ID NO: 467) was used to measure RNA. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®.

Certain sequences complementary to the STAT3 mouse gene sequence showed good inhibition in b.END cells. Results are presented in Table 3 as percent inhibition of STAT3, relative to untreated control cells. The human oligonucleotides in Table 3 were compared to the mouse STAT-3 genomic sequence, designated herein as SEQ ID NO: 3 (the complement of GENBANK Accession No. NT_165773.2 truncated from nucleotides 12286001 to 12344000). "Mouse Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the murine sequence. "Mouse Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted murine sequence.

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Table 3

Inhibition of human STAT3 mRNA levels by certain cEt chimeric antisense oligonucleotides complementary to SEQ ID NO: 1 and SEQ ID NO: 3

ISIS NO	Mouse Start Site	Mouse Stop Site	% inhibition	SEQ ID NO
481549	5283	5298	96	413
481553	9913	9928	94	421
481768	3189	3202	91	402
481356	30356	30371	83	21
481548	4045	4060	82	411
481554	14662	14677	82	423
481426	48328	48343	82	165
481580	30333	30346	81	20
481412	47413	47428	81	135
481417	47636	47651	81	147
481418	47637	47652	80	148
481355	30332	30347	79	19
481396	43120	43135	79	443
481416	47634	47649	79	144
481420	47681	47696	79	153
481358	32842	32857	78	25
481363	33520	33535	78	35
481570	51870	51885	78	457
481382	37857	37872	77	74
481378	36560	36575	76	66
481431	48403	48418	76	175
481453	53034	53049	76	223
481621	43121	43134	75	444
481641	47635	47648	75	145
481637	47414	47427	74	136
481380	36631	36646	73	70
481574	53000	53015	73	221
481601	36392	36405	71	62
481419	47638	47653	71	150
481371	35938	35953	70	51
481642	47637	47650	70	149
481542	3180	3195	69	399
481547	3313	3328	69	409
481772	3314	3327	69	410
481362	32929	32944	69	33
481653	48362	48375	69	170
481786	38812	38825	68	440

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481415	47629	47644	68	141
481543	3188	3203	67	401
481793	51714	51727	67	454
481443	52060	52075	67	200
481684	53229	53242	67	236
481398	45226	45241	66	106
481560	36394	36409	65	438
481643	47638	47651	65	151
481430	48387	48402	65	173
481440	52024	52039	65	194

Example 3: Tolerability of antisense oligonucleotides targeting STAT3 in BALB/c mice

Forty antisense oligonucleotides exhibiting a high level of potency, selected from among the 452 compounds evaluated in Example 1, were further tested for *in vivo* tolerability.

5 Groups of 2-4 male BALB/c mice were injected subcutaneously twice a week for 3 weeks with 25 mg/kg of ISIS antisense oligonucleotides. One group of 4 male BALB/c mice was injected subcutaneously twice a week for 3 weeks with PBS. This group of mice was utilized as a control group to which the treatment groups were compared. One day after the last dose, body weights were taken, mice were euthanized, and organs and plasma were harvested for further analysis.

10 The body weights of the mice were measured pre-dose and at the end of the treatment period. Percent increase over the initial body weight was calculated. Liver, spleen, and kidney weights were measured at the end of the study and were compared to PBS treated mice.

To evaluate the effect of ISIS oligonucleotides on metabolic function, plasma concentrations of transaminases and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase), AST (aspartate transaminase), and BUN were measured.

15

Among the forty antisense oligonucleotides tested, certain antisense oligonucleotides, including ISIS 481374, ISIS 481390, ISIS 481420, ISIS 481431, ISIS 481453, ISIS 481464, ISIS 481475, ISIS 481495, ISIS 481500, ISIS 481501, ISIS 481525, ISIS 481548, ISIS 481549, ISIS 481597, ISIS 481695, ISIS 481700, ISIS 481702, ISIS 481710, ISIS 481725, ISIS 481750, and ISIS 481763 met tolerability thresholds for body weight, organ weight, ALT, AST, and BUN parameters.

20

Example 4: Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

Gapmers from Examples 1 and 2 exhibiting significant *in vitro* inhibition of STAT3 were tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using

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electroporation with 31.25 nM, 62.5 nM, 125 nM, 250 nM, 500 nM, and 1,0000 nM concentrations of antisense oligonucleotide, as specified in Table 4. After a treatment period of approximately 16 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199 (forward sequence ACATGCCACTTTGGTGTTCATAA, designated
 5 herein as SEQ ID NO: 6; reverse sequence TCTTCGTAGATTGTGCTGATAGAGAAC, designated herein as SEQ ID NO: 7; probe sequence CAGTATAGCCGCTTCCTGCAAGAGTCGAA, designated herein as SEQ ID NO: 8) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

10 The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 4 and was calculated by plotting the concentrations of oligonucleotides used versus the percent inhibition of STAT3 mRNA expression achieved at each concentration and noting the concentration of oligonucleotide at which 50% inhibition of STAT3 mRNA expression was achieved compared to the control. As illustrated in Table 4, STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense
 15 oligonucleotide treated cells.

Table 4
Dose-dependent antisense inhibition of human STAT3 in HuVEC cells using electroporation

ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	IC ₅₀ (μM)
481355	19	15	36	61	75	89	0.18
481374	25	42	52	72	82	88	0.10
481390	17	37	44	60	73	86	0.15
481420	23	20	40	60	81	92	0.16
481453	21	37	52	69	79	88	0.12
481464	57	73	81	90	94	94	<0.03
481475	22	46	54	78	83	92	0.10
481500	25	37	42	75	83	90	0.12
481501	32	57	69	82	94	94	0.05
481523	35	60	74	85	90	93	0.04
481525	36	53	60	79	89	92	0.06
481549	0	16	60	81	90	96	0.15
481554	0	15	28	49	70	86	0.25
481597	8	18	39	48	64	83	0.24
481695	15	27	39	50	64	80	0.22
481700	0	17	44	58	80	88	0.20
481710	12	39	65	79	86	90	0.11
481715	11	26	32	44	53	69	0.36
481725	27	40	56	77	89	93	0.09

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481750	7	24	46	63	83	89	0.16
481755	17	28	30	54	68	80	0.20
481768	7	21	27	44	67	85	0.26

Example 5: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in SK-BR-3 cells

Gapmers from Example 4 were tested at various doses in SK-BR-3 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated with 0.02 μ M, 0.1 μ M, 0.5 μ M, 1 μ M, 2.5 μ M, and 10 μ M concentrations of antisense oligonucleotide, as specified in Table 5. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 5. As illustrated in Table 5, most of the ISIS oligonucleotides were able to penetrate the cell membrane and STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 5
Dose-dependent antisense inhibition of human STAT3 by free-uptake of ISIS oligonucleotide by SK-BR-3 cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	1 μ M	2.5 μ M	10 μ M	IC ₅₀ (μ M)
481374	10	18	18	16	8	25	15.9
481390	0	10	11	12	40	72	3.2
481453	14	13	27	45	58	79	1.3
481464	23	32	57	70	85	93	0.5
481475	0	0	35	49	72	88	1.0
481500	7	9	26	45	49	75	1.7
481501	0	0	4	5	53	65	2.7
481523	9	24	56	67	83	92	0.5
481525	0	17	13	15	32	68	4.4
481549	0	0	0	16	33	54	8.2
481597	1	0	11	14	22	44	10.6
481710	5	0	10	13	27	66	6.0
481725	29	45	47	39	39	63	2.6
481750	19	24	36	42	71	80	1.1
481763	30	38	51	63	81	89	0.6
481768	12	5	34	25	32	35	12.4

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Example 6: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in U251-MG cells

Gapmers from Example 5 were further tested at various doses in U251-MG cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated with 0.02 μ M, 0.1 μ M, 0.5 μ M, 1 μ M, 2.5 μ M, and 10 μ M concentrations of antisense oligonucleotide, as specified in Table 6. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 6. As illustrated in Table 6, most of the ISIS oligonucleotides were able to penetrate the cell membrane and STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 6
Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by U251-MG cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	1 μ M	2.5 μ M	10 μ M	IC ₅₀ (μ M)
481374	0	0	10	0	12	25	15.7
481390	0	4	10	8	16	31	13.9
481453	4	3	15	16	20	42	11.0
481464	13	11	41	42	54	79	1.3
481475	3	13	26	37	41	67	2.6
481500	2	12	14	12	25	38	11.7
481501	0	0	2	1	14	47	10.3
481523	22	27	39	45	63	83	1.1
481525	1	1	17	17	35	60	6.3
481549	0	0	0	0	9	29	14.5
481597	3	3	12	18	18	47	10.1
481695	0	14	12	22	25	33	12.9
481710	0	0	0	0	6	23	16.8
481725	0	0	5	7	20	38	11.8
481750	4	15	18	18	17	33	13.2
481763	15	16	25	36	36	64	3.2
481768	22	16	18	22	21	37	12.2

BIOL0142WO**Example 7: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in U251-MG cells**

ISIS 481464 and ISIS 481549, from the studies described above, were further tested at different doses in U251-MG cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated with 0.1 μ M, 1 μ M, 5 μ M, 10 μ M, and 20 μ M concentrations of antisense oligonucleotide, as specified in Table 7. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 7. As illustrated in Table 7, both the ISIS oligonucleotides were able to penetrate the cell membrane.

Table 7
Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by U251-MG cells

ISIS No	0.1 μ M	1 μ M	5 μ M	10 μ M	20 μ M	IC ₅₀ (μ M)
481464	0	30	69	80	79	2.3
481549	0	0	26	35	38	>20

Example 8: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in MDA-MB-231 cells

ISIS 481464 and ISIS 481549 were further tested at different doses in MDA-MB-231 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated with 0.02 μ M, 0.2 μ M, 1.0 μ M, 5.0 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 8. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 8. As illustrated in Table 8, both the ISIS oligonucleotides were able to penetrate the cell membrane and significantly reduce STAT3 mRNA levels in a dose-dependent manner.

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Table 8

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by MDA-MB-231 cells

ISIS No	0.02 μ M	0.2 μ M	1.0 μ M	5.0 μ M	10.0 μ M	IC ₅₀ (μ M)
481464	0	25	71	85	87	0.6
481549	0	2	33	49	66	4.4

Example 9: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in A431 cells

ISIS 481464 and ISIS 481549 were further tested at different doses in A431 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated with 0.02 μ M, 0.2 μ M, 1.0 μ M, 5.0 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 9. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 9. As illustrated in Table 9, both the ISIS oligonucleotides were able to penetrate the cell membrane and significantly reduce STAT3 mRNA levels in a dose-dependent manner.

Table 9

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by A431 cells

ISIS No	0.02 μ M	0.2 μ M	1.0 μ M	5.0 μ M	10.0 μ M	IC ₅₀ (μ M)
481464	79	93	98	98	98	<0.02
481549	0	38	68	82	84	0.6

Example 10: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in H460 cells

ISIS 481464 and ISIS 481549 were further tested at different doses in H460 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated with 0.02 μ M, 0.2 μ M, 1.0 μ M, 5.0 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 10. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

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The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 10. As illustrated in Table 10, both the ISIS oligonucleotides were able to penetrate the cell membrane and significantly reduce STAT3 mRNA levels in a dose-dependent manner.

Table 10

5 Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by H460 cells

ISIS No	0.02 μ M	0.2 μ M	1.0 μ M	5.0 μ M	10.0 μ M	IC ₅₀ (μ M)
481464	46	89	96	97	98	0.01
481549	8	53	78	96	98	0.23

Example 11: Antisense inhibition of human STAT3 in HuVEC cells

Antisense oligonucleotides were designed targeting a human STAT3 nucleic acid and were tested for
 10 their effect on human STAT3 mRNA expression *in vitro*. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS199 (forward sequence
 ACATGCCACTTTGGTGTTCATAA, designated herein as SEQ ID NO: 6; reverse sequence
 15 TCTTCGTAGATTGTGCTGATAGAGAAC, designated herein as SEQ ID NO: 7; probe sequence
 CAGTATAGCCGCTTCCTGCAAGAGTCGAA, designated herein as SEQ ID NO: 8) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The chimeric antisense oligonucleotides in Table 11 were designed as 3-10-3 MOE, deoxy, and cEt
 20 gapmers. The gapmers are 16 nucleotides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three nucleosides each. Each nucleoside in the 5'-wing segment has a 2'-MOE sugar modification. Each nucleoside in the 3'-wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5'-
 25 methylcytosines. The chemistry column of Table 11 presents the sugar motif of each gapmer, wherein 'e' indicates a 2'-MOE nucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'd' indicates a 2'-deoxynucleoside.

"Human Target start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Human Target stop site" indicates the 3'-most nucleoside to which the gapmer is
 30 targeted in the human gene sequence. Each gapmer listed in Table 11 is targeted to human STAT3 mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_139276.2).

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Table 11

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

Human Start Site	Human Stop Site	ISIS No	Sequence	Chemistry	% inhibition	SEQ ID NO
1	16	528170	CGCAGCTCCGGAAC	e-e-e-d ₍₁₀₎ -k-k-k	12	471
2	17	528171	CCGCAGCTCCGGAAC	e-e-e-d ₍₁₀₎ -k-k-k	11	472
4	19	528172	CGCCGCAGCTCCGGA	e-e-e-d ₍₁₀₎ -k-k-k	10	473
5	20	528173	CCGCCGCAGCTCCGGA	e-e-e-d ₍₁₀₎ -k-k-k	22	474
32	47	528174	ACCCCCGGCTCCCCCT	e-e-e-d ₍₁₀₎ -k-k-k	18	475
34	49	528175	GAACCCCCGGCTCCCC	e-e-e-d ₍₁₀₎ -k-k-k	17	476
35	50	528176	GGAACCCCCGGCTCCC	e-e-e-d ₍₁₀₎ -k-k-k	23	477
36	51	528177	CGGAACCCCCGGCTCC	e-e-e-d ₍₁₀₎ -k-k-k	15	478
38	53	528178	GTCGGAACCCCCGGCT	e-e-e-d ₍₁₀₎ -k-k-k	21	479
39	54	528179	CGTCGGAACCCCCGGC	e-e-e-d ₍₁₀₎ -k-k-k	19	480
57	72	528180	TTGTTCCCTCGGCTGC	e-e-e-d ₍₁₀₎ -k-k-k	40	481
58	73	528181	CTTGTTCCCTCGGCTG	e-e-e-d ₍₁₀₎ -k-k-k	28	482
60	75	528182	GGCTTGTTCCCTCGGC	e-e-e-d ₍₁₀₎ -k-k-k	25	483
61	76	528183	GGGCTTGTTCCCTCGG	e-e-e-d ₍₁₀₎ -k-k-k	34	484
75	90	528184	CCAGGATCCGGTTGGG	e-e-e-d ₍₁₀₎ -k-k-k	34	485
76	91	528185	TCCAGGATCCGGTTGG	e-e-e-d ₍₁₀₎ -k-k-k	15	9
77	92	528186	GTCCAGGATCCGGTTG	e-e-e-d ₍₁₀₎ -k-k-k	28	486
78	93	528187	TGTCCAGGATCCGGTT	e-e-e-d ₍₁₀₎ -k-k-k	27	487
79	94	528188	CTGTCCAGGATCCGGT	e-e-e-d ₍₁₀₎ -k-k-k	33	488
81	96	528189	GCCTGTCCAGGATCCG	e-e-e-d ₍₁₀₎ -k-k-k	63	489
83	98	528190	GTGCCTGTCCAGGATC	e-e-e-d ₍₁₀₎ -k-k-k	36	490
189	204	528191	AGAGGCCGAGAGGCCG	e-e-e-d ₍₁₀₎ -k-k-k	2	491
210	225	528192	GGTCCCAACTGTTTCT	e-e-e-d ₍₁₀₎ -k-k-k	11	492
232	247	528193	GGGCCATCCTGCTAAA	e-e-e-d ₍₁₀₎ -k-k-k	14	493
233	248	528194	TGGGCCATCCTGCTAA	e-e-e-d ₍₁₀₎ -k-k-k	16	494
234	249	528195	TTGGGCCATCCTGCTA	e-e-e-d ₍₁₀₎ -k-k-k	9	495
236	251	528196	CATTGGGCCATCCTGC	e-e-e-d ₍₁₀₎ -k-k-k	39	496
237	252	528197	CCATTGGGCCATCCTG	e-e-e-d ₍₁₀₎ -k-k-k	38	497
239	254	528198	TTCCATTGGGCCATCC	e-e-e-d ₍₁₀₎ -k-k-k	19	498
240	255	528199	ATTCCATTGGGCCATC	e-e-e-d ₍₁₀₎ -k-k-k	27	15
244	259	528200	GCTGATTCCATTGGGC	e-e-e-d ₍₁₀₎ -k-k-k	18	500
245	260	528201	AGCTGATTCCATTGGG	e-e-e-d ₍₁₀₎ -k-k-k	20	501
246	261	528202	TAGCTGATTCCATTGG	e-e-e-d ₍₁₀₎ -k-k-k	41	502
247	262	528203	GTAGCTGATTCCATTG	e-e-e-d ₍₁₀₎ -k-k-k	37	503
250	265	528204	GCTGTAGCTGATTCCA	e-e-e-d ₍₁₀₎ -k-k-k	83	504
251	266	528205	TGCTGTAGCTGATTCC	e-e-e-d ₍₁₀₎ -k-k-k	72	505
252	267	528206	CTGCTGTAGCTGATTC	e-e-e-d ₍₁₀₎ -k-k-k	44	506
253	268	528207	GCTGCTGTAGCTGATT	e-e-e-d ₍₁₀₎ -k-k-k	49	507

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263	278	528208	CGTGTGTCAAGCTGCT	e-e-e-d ₍₁₀₎ -k-k-k	73	508
264	279	528209	CCGTGTGTCAAGCTGC	e-e-e-d ₍₁₀₎ -k-k-k	81	17
265	280	528210	ACCGTGTGTCAAGCTG	e-e-e-d ₍₁₀₎ -k-k-k	78	509
266	281	528211	TACCGTGTGTCAAGCT	e-e-e-d ₍₁₀₎ -k-k-k	72	510
267	282	528212	GTACCGTGTGTCAAGC	e-e-e-d ₍₁₀₎ -k-k-k	81	511
268	283	528213	GGTACCGTGTGTCAAG	e-e-e-d ₍₁₀₎ -k-k-k	46	512
270	285	528214	CAGGTACCGTGTGTCA	e-e-e-d ₍₁₀₎ -k-k-k	80	513
271	286	528215	CCAGGTACCGTGTGTG	e-e-e-d ₍₁₀₎ -k-k-k	69	514
272	287	528216	TCCAGGTACCGTGTGT	e-e-e-d ₍₁₀₎ -k-k-k	41	515
273	288	528217	CTCCAGGTACCGTGTG	e-e-e-d ₍₁₀₎ -k-k-k	44	516
274	289	528218	GCTCCAGGTACCGTGT	e-e-e-d ₍₁₀₎ -k-k-k	32	517
275	290	528219	TGCTCCAGGTACCGTG	e-e-e-d ₍₁₀₎ -k-k-k	50	518
291	306	528220	GTAGAGCTGATGGAGC	e-e-e-d ₍₁₀₎ -k-k-k	12	519
292	307	528221	TGTAGAGCTGATGGAG	e-e-e-d ₍₁₀₎ -k-k-k	0	520
295	310	528222	CACTGTAGAGCTGATG	e-e-e-d ₍₁₀₎ -k-k-k	0	521
297	312	528223	GTCAGTGTAGAGCTGA	e-e-e-d ₍₁₀₎ -k-k-k	44	522
302	317	528224	AAGCTGTCACTGTAGA	e-e-e-d ₍₁₀₎ -k-k-k	20	523
303	318	528225	GAAGCTGTCACTGTAG	e-e-e-d ₍₁₀₎ -k-k-k	24	524
307	322	528226	TTGGGAAGCTGTCACT	e-e-e-d ₍₁₀₎ -k-k-k	35	525
308	323	528227	ATTGGGAAGCTGTAC	e-e-e-d ₍₁₀₎ -k-k-k	29	526
310	325	528228	CCATTGGGAAGCTGTC	e-e-e-d ₍₁₀₎ -k-k-k	33	527
322	337	519639	ACTGCCGCAGCTCCAT	e-e-e-d ₍₁₀₎ -k-k-k	37	19
329	344	528229	GCCAGAACTGCCGCA	e-e-e-d ₍₁₀₎ -k-k-k	20	528
330	345	528230	GGCCAGAACTGCCGC	e-e-e-d ₍₁₀₎ -k-k-k	1	529
331	346	528231	GGGCCAGAACTGCCG	e-e-e-d ₍₁₀₎ -k-k-k	1	530
345	360	528232	ACTCTCAATCCAAGGG	e-e-e-d ₍₁₀₎ -k-k-k	14	531
346	361	528233	GACTCTCAATCCAAGG	e-e-e-d ₍₁₀₎ -k-k-k	10	21
347	362	528234	TGACTCTCAATCCAAG	e-e-e-d ₍₁₀₎ -k-k-k	6	532
351	366	528235	ATCTTGACTCTCAATC	e-e-e-d ₍₁₀₎ -k-k-k	38	533
353	368	528236	CAATCTTGACTCTCAA	e-e-e-d ₍₁₀₎ -k-k-k	29	534
354	369	528237	CCAATCTTGACTCTCA	e-e-e-d ₍₁₀₎ -k-k-k	60	535
355	370	528238	CCCAATCTTGACTCTC	e-e-e-d ₍₁₀₎ -k-k-k	37	536
356	371	528239	GCCCAATCTTGACTCT	e-e-e-d ₍₁₀₎ -k-k-k	48	537
357	372	528240	TGCCCAATCTTGACTC	e-e-e-d ₍₁₀₎ -k-k-k	40	538
358	373	528241	ATGCCCAATCTTGACT	e-e-e-d ₍₁₀₎ -k-k-k	21	539
359	374	528242	TATGCCCAATCTTGAC	e-e-e-d ₍₁₀₎ -k-k-k	27	540
362	377	528243	GCATATGCCCAATCTT	e-e-e-d ₍₁₀₎ -k-k-k	16	541
363	378	528244	CGCATATGCCCAATCT	e-e-e-d ₍₁₀₎ -k-k-k	50	542
367	382	528245	TGGCCGCATATGCCCA	e-e-e-d ₍₁₀₎ -k-k-k	67	543
368	383	528246	CTGGCCGCATATGCC	e-e-e-d ₍₁₀₎ -k-k-k	47	544
369	384	528247	GCTGGCCGCATATGCC	e-e-e-d ₍₁₀₎ -k-k-k	54	545
370	385	528248	TGCTGGCCGCATATGC	e-e-e-d ₍₁₀₎ -k-k-k	35	546

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371	386	528249	TTGCTGGCCGCATATG	e-e-e-d ₍₁₀₎ -k-k-k	22	547
372	387	528250	TTTGCTGGCCGCATAT	e-e-e-d ₍₁₀₎ -k-k-k	19	548
373	388	528251	CTTTGCTGGCCGCATA	e-e-e-d ₍₁₀₎ -k-k-k	27	549
374	389	528252	TCTTTGCTGGCCGCAT	e-e-e-d ₍₁₀₎ -k-k-k	34	550
375	390	528253	TTCTTTGCTGGCCGCA	e-e-e-d ₍₁₀₎ -k-k-k	59	23
376	391	528254	ATTCTTTGCTGGCCGC	e-e-e-d ₍₁₀₎ -k-k-k	63	551
378	393	528255	TGATTCTTTGCTGGCC	e-e-e-d ₍₁₀₎ -k-k-k	30	552
379	394	528256	GTGATTCTTTGCTGGC	e-e-e-d ₍₁₀₎ -k-k-k	47	553
383	398	528257	GCATGTGATTCTTTGC	e-e-e-d ₍₁₀₎ -k-k-k	43	554
384	399	528258	GGCATGTGATTCTTTG	e-e-e-d ₍₁₀₎ -k-k-k	47	555
388	403	528259	AAGTGGCATGTGATTC	e-e-e-d ₍₁₀₎ -k-k-k	43	556
391	406	528260	CCAAAGTGGCATGTGA	e-e-e-d ₍₁₀₎ -k-k-k	46	557
393	408	528261	CACCAAAGTGGCATGT	e-e-e-d ₍₁₀₎ -k-k-k	32	558
395	410	528262	AACACCAAAGTGGCAT	e-e-e-d ₍₁₀₎ -k-k-k	41	559
397	412	528263	GAAACACCAAAGTGGC	e-e-e-d ₍₁₀₎ -k-k-k	69	560
427	442	528264	ACTGCTGGTCAATCTC	e-e-e-d ₍₁₀₎ -k-k-k	27	561
428	443	528265	TACTGCTGGTCAATCT	e-e-e-d ₍₁₀₎ -k-k-k	32	562
430	445	528266	TATACTGCTGGTCAAT	e-e-e-d ₍₁₀₎ -k-k-k	27	563
431	446	528267	CTATACTGCTGGTCAA	e-e-e-d ₍₁₀₎ -k-k-k	38	564
432	447	528268	GCTATACTGCTGGTCA	e-e-e-d ₍₁₀₎ -k-k-k	58	565
433	448	528269	GGCTATACTGCTGGTC	e-e-e-d ₍₁₀₎ -k-k-k	69	566
434	449	528270	CGGCTATACTGCTGGT	e-e-e-d ₍₁₀₎ -k-k-k	73	567
435	450	528271	GCGGCTATACTGCTGG	e-e-e-d ₍₁₀₎ -k-k-k	71	568
436	451	528272	AGCGGCTATACTGCTG	e-e-e-d ₍₁₀₎ -k-k-k	54	569
437	452	528273	AAGCGGCTATACTGCT	e-e-e-d ₍₁₀₎ -k-k-k	36	570
439	454	528274	GGAAGCGGCTATACTG	e-e-e-d ₍₁₀₎ -k-k-k	27	571
440	455	528275	AGGAAGCGGCTATACT	e-e-e-d ₍₁₀₎ -k-k-k	21	572
441	456	528276	CAGGAAGCGGCTATAC	e-e-e-d ₍₁₀₎ -k-k-k	12	573
442	457	528277	GCAGGAAGCGGCTATA	e-e-e-d ₍₁₀₎ -k-k-k	14	574
443	458	528278	TGCAGGAAGCGGCTAT	e-e-e-d ₍₁₀₎ -k-k-k	21	575
444	459	528279	TTGCAGGAAGCGGCTA	e-e-e-d ₍₁₀₎ -k-k-k	31	576
445	460	528280	CTTGCAGGAAGCGGCT	e-e-e-d ₍₁₀₎ -k-k-k	44	577
463	478	528281	GATAGAGAACATTCGA	e-e-e-d ₍₁₀₎ -k-k-k	25	578
464	479	528282	TGATAGAGAACATTCG	e-e-e-d ₍₁₀₎ -k-k-k	39	579
469	484	528283	TGTGCTGATAGAGAAC	e-e-e-d ₍₁₀₎ -k-k-k	41	580
471	486	528284	ATTGTGCTGATAGAGA	e-e-e-d ₍₁₀₎ -k-k-k	38	581
472	487	528285	GATTGTGCTGATAGAG	e-e-e-d ₍₁₀₎ -k-k-k	50	582
473	488	528286	AGATTGTGCTGATAGA	e-e-e-d ₍₁₀₎ -k-k-k	49	583
475	490	528287	GTAGATTGTGCTGATA	e-e-e-d ₍₁₀₎ -k-k-k	14	584
476	491	528288	CGTAGATTGTGCTGAT	e-e-e-d ₍₁₀₎ -k-k-k	8	585
490	505	528289	ACTGCTTGATTCTTCG	e-e-e-d ₍₁₀₎ -k-k-k	9	33
511	526	528290	CAAGATACCTGCTCTG	e-e-e-d ₍₁₀₎ -k-k-k	48	35

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512	527	528291	TCAAGATACCTGCTCT	e-e-e-d ₍₁₀₎ -k-k-k	34	586
513	528	528292	CTCAAGATACCTGCTC	e-e-e-d ₍₁₀₎ -k-k-k	19	587
514	529	528293	TCTCAAGATACCTGCT	e-e-e-d ₍₁₀₎ -k-k-k	31	588
517	532	528294	GCTTCTCAAGATACCT	e-e-e-d ₍₁₀₎ -k-k-k	42	589
519	534	528295	TGGCTTCTCAAGATAC	e-e-e-d ₍₁₀₎ -k-k-k	37	590
522	537	528296	CATTGGCTTCTCAAGA	e-e-e-d ₍₁₀₎ -k-k-k	11	591
523	538	528297	CCATTGGCTTCTCAAG	e-e-e-d ₍₁₀₎ -k-k-k	23	592
530	545	528298	GCAATCTCCATTGGCT	e-e-e-d ₍₁₀₎ -k-k-k	46	593
531	546	528299	GGCAATCTCCATTGGC	e-e-e-d ₍₁₀₎ -k-k-k	37	594
532	547	528300	GGGCAATCTCCATTGG	e-e-e-d ₍₁₀₎ -k-k-k	24	595
533	548	528301	CGGGCAATCTCCATTG	e-e-e-d ₍₁₀₎ -k-k-k	15	596
534	549	528302	CCGGGCAATCTCCATT	e-e-e-d ₍₁₀₎ -k-k-k	30	597
535	550	528303	TCCGGGCAATCTCCAT	e-e-e-d ₍₁₀₎ -k-k-k	29	598
536	551	528304	ATCCGGGCAATCTCCA	e-e-e-d ₍₁₀₎ -k-k-k	32	599
537	552	528305	AATCCGGGCAATCTCC	e-e-e-d ₍₁₀₎ -k-k-k	32	600
538	553	528306	CAATCCGGGCAATCTC	e-e-e-d ₍₁₀₎ -k-k-k	24	601
539	554	528307	ACAATCCGGGCAATCT	e-e-e-d ₍₁₀₎ -k-k-k	21	602
540	555	528308	CACAATCCGGGCAATC	e-e-e-d ₍₁₀₎ -k-k-k	14	603
541	556	528309	CCACAATCCGGGCAAT	e-e-e-d ₍₁₀₎ -k-k-k	13	604
543	558	528310	GGCCACAATCCGGGCA	e-e-e-d ₍₁₀₎ -k-k-k	27	605
546	561	528311	CCGGGCCACAATCCGG	e-e-e-d ₍₁₀₎ -k-k-k	27	606
547	562	528312	ACCGGGCCACAATCCG	e-e-e-d ₍₁₀₎ -k-k-k	58	607
548	563	528313	CACCGGGCCACAATCC	e-e-e-d ₍₁₀₎ -k-k-k	25	608
549	564	528314	GCACCGGGCCACAATC	e-e-e-d ₍₁₀₎ -k-k-k	18	609
550	565	528315	GGCACCGGGCCACAAT	e-e-e-d ₍₁₀₎ -k-k-k	33	610
551	566	528316	AGGCACCGGGCCACAA	e-e-e-d ₍₁₀₎ -k-k-k	42	611
558	573	528317	TTCCCACAGGCACCGG	e-e-e-d ₍₁₀₎ -k-k-k	47	612
586	601	528318	TGGCTGCAGTCTGTAG	e-e-e-d ₍₁₀₎ -k-k-k	12	613
592	607	528319	CCGCAGTGGCTGCAGT	e-e-e-d ₍₁₀₎ -k-k-k	10	614
599	614	528320	TGCTGGGCCGCAGTGG	e-e-e-d ₍₁₀₎ -k-k-k	14	615
601	616	528321	CTTGCTGGGCCGCAGT	e-e-e-d ₍₁₀₎ -k-k-k	0	616
603	618	528322	CCCTTGCTGGGCCGCA	e-e-e-d ₍₁₀₎ -k-k-k	6	617
604	619	528323	CCCCTTGCTGGGCCGC	e-e-e-d ₍₁₀₎ -k-k-k	21	618
605	620	528324	CCCCCTTGCTGGGCCG	e-e-e-d ₍₁₀₎ -k-k-k	8	619
608	623	528325	TGGCCCCCTTGCTGGG	e-e-e-d ₍₁₀₎ -k-k-k	0	620
615	630	528326	GTTGGCCTGGCCCCCT	e-e-e-d ₍₁₀₎ -k-k-k	31	621
616	631	528327	GGTTGGCCTGGCCCCC	e-e-e-d ₍₁₀₎ -k-k-k	47	622
617	632	528328	TGGTTGGCCTGGCCCC	e-e-e-d ₍₁₀₎ -k-k-k	36	623
646	661	528329	GCTTCTCCGTCACCAC	e-e-e-d ₍₁₀₎ -k-k-k	28	624
647	662	528330	TGCTTCTCCGTCACCA	e-e-e-d ₍₁₀₎ -k-k-k	22	625
649	664	528331	GCTGCTTCTCCGTCAC	e-e-e-d ₍₁₀₎ -k-k-k	35	626
667	682	528332	GGTGCTGCTCCAGCAT	e-e-e-d ₍₁₀₎ -k-k-k	21	627

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678	693	528333	GACATCCTGAAGGTGC	e-e-e-d ₍₁₀₎ -k-k-k	0	628
682	697	528334	TCCGGACATCCTGAAG	e-e-e-d ₍₁₀₎ -k-k-k	1	629
683	698	528335	TTCCGGACATCCTGAA	e-e-e-d ₍₁₀₎ -k-k-k	0	630
684	699	528336	CTTCCGGACATCCTGA	e-e-e-d ₍₁₀₎ -k-k-k	0	631
685	700	528337	TCTTCCGGACATCCTG	e-e-e-d ₍₁₀₎ -k-k-k	0	632
686	701	528338	CTCTTCCGGACATCCT	e-e-e-d ₍₁₀₎ -k-k-k	19	633
687	702	528339	TCTCTTCCGGACATCC	e-e-e-d ₍₁₀₎ -k-k-k	21	634
688	703	528340	CTCTCTTCCGGACATC	e-e-e-d ₍₁₀₎ -k-k-k	17	635
689	704	528341	ACTCTCTTCCGGACAT	e-e-e-d ₍₁₀₎ -k-k-k	37	636
727	742	528342	GATTCTCTACCACTTT	e-e-e-d ₍₁₀₎ -k-k-k	33	637
730	745	528343	GGAGATTCTCTACCAC	e-e-e-d ₍₁₀₎ -k-k-k	40	53
731	746	528344	TGGAGATTCTCTACCA	e-e-e-d ₍₁₀₎ -k-k-k	32	638
732	747	528345	CTGGAGATTCTCTACC	e-e-e-d ₍₁₀₎ -k-k-k	18	639
733	748	528346	CCTGGAGATTCTCTAC	e-e-e-d ₍₁₀₎ -k-k-k	12	640
738	753	528347	GTCATCCTGGAGATTC	e-e-e-d ₍₁₀₎ -k-k-k	54	641
764	779	528348	TTGAGGGTTTATAGT	e-e-e-d ₍₁₀₎ -k-k-k	0	642
775	790	528349	CTCCTTGACTCTTGAG	e-e-e-d ₍₁₀₎ -k-k-k	21	643
781	796	528350	GCATGTCTCCTTGACT	e-e-e-d ₍₁₀₎ -k-k-k	29	644
782	797	528351	TGCATGTCTCCTTGAC	e-e-e-d ₍₁₀₎ -k-k-k	30	645
783	798	528352	TTGCATGTCTCCTTGA	e-e-e-d ₍₁₀₎ -k-k-k	17	646
787	802	528353	GATCTTGATGTCTCC	e-e-e-d ₍₁₀₎ -k-k-k	61	647
788	803	518346	AGATCTTGATGTCTC	e-e-e-d ₍₁₀₎ -k-k-k	36	57
790	805	528354	TCAGATCTTGATGTC	e-e-e-d ₍₁₀₎ -k-k-k	43	648
792	807	528355	ATTCAGATCTTGATG	e-e-e-d ₍₁₀₎ -k-k-k	9	649
794	809	528356	CCATTCAGATCTTGCA	e-e-e-d ₍₁₀₎ -k-k-k	37	650
795	810	528357	TCCATTCAGATCTTGC	e-e-e-d ₍₁₀₎ -k-k-k	55	651
796	811	528358	TTCCATTCAGATCTTG	e-e-e-d ₍₁₀₎ -k-k-k	17	652
803	818	528359	TGGTTGTTTCCATTCA	e-e-e-d ₍₁₀₎ -k-k-k	33	653
804	819	528360	CTGGTTGTTTCCATTC	e-e-e-d ₍₁₀₎ -k-k-k	18	654
806	821	528361	GACTGGTTGTTTCCAT	e-e-e-d ₍₁₀₎ -k-k-k	23	655
807	822	528362	TGACTGGTTGTTTCCA	e-e-e-d ₍₁₀₎ -k-k-k	33	656
813	828	528363	GGTCACTGACTGGTTG	e-e-e-d ₍₁₀₎ -k-k-k	43	657
814	829	528364	TGGTCACTGACTGGTT	e-e-e-d ₍₁₀₎ -k-k-k	62	658
848	863	528365	GTGAGCATCTGTCCA	e-e-e-d ₍₁₀₎ -k-k-k	41	659
852	867	528366	CGCAGTGAGCATCTGT	e-e-e-d ₍₁₀₎ -k-k-k	0	660
853	868	528367	GCGCAGTGAGCATCTG	e-e-e-d ₍₁₀₎ -k-k-k	0	661
854	869	528368	AGCGCAGTGAGCATCT	e-e-e-d ₍₁₀₎ -k-k-k	7	662
855	870	528369	CAGCGCAGTGAGCATC	e-e-e-d ₍₁₀₎ -k-k-k	6	663
857	872	528370	TCCAGCGCAGTGAGCA	e-e-e-d ₍₁₀₎ -k-k-k	12	664
858	873	528371	GTCCAGCGCAGTGAGC	e-e-e-d ₍₁₀₎ -k-k-k	11	665
859	874	528372	GGTCCAGCGCAGTGAG	e-e-e-d ₍₁₀₎ -k-k-k	8	666
860	875	528373	TGGTCCAGCGCAGTGA	e-e-e-d ₍₁₀₎ -k-k-k	12	667

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862	877	528374	TCTGGTCCAGCGCAGT	e-e-e-d ₍₁₀₎ -k-k-k	9	668
863	878	528375	ATCTGGTCCAGCGCAG	e-e-e-d ₍₁₀₎ -k-k-k	8	669
864	879	528376	CATCTGGTCCAGCGCA	e-e-e-d ₍₁₀₎ -k-k-k	0	670
865	880	528377	GCATCTGGTCCAGCGC	e-e-e-d ₍₁₀₎ -k-k-k	28	671
867	882	528378	CCGCATCTGGTCCAGC	e-e-e-d ₍₁₀₎ -k-k-k	72	672
868	883	528379	TCCGCATCTGGTCCAG	e-e-e-d ₍₁₀₎ -k-k-k	43	61
869	884	528380	CTCCGCATCTGGTCCA	e-e-e-d ₍₁₀₎ -k-k-k	34	673
870	885	528381	TCTCCGCATCTGGTCC	e-e-e-d ₍₁₀₎ -k-k-k	42	674
871	886	528382	TTCTCCGCATCTGGTC	e-e-e-d ₍₁₀₎ -k-k-k	37	675
872	887	528383	CTTCTCCGCATCTGGT	e-e-e-d ₍₁₀₎ -k-k-k	23	676
873	888	528384	GCTTCTCCGCATCTGG	e-e-e-d ₍₁₀₎ -k-k-k	36	677
875	890	528385	ATGCTTCTCCGCATCT	e-e-e-d ₍₁₀₎ -k-k-k	45	678
876	891	528386	GATGCTTCTCCGCATC	e-e-e-d ₍₁₀₎ -k-k-k	14	679
877	892	528387	CGATGCTTCTCCGCAT	e-e-e-d ₍₁₀₎ -k-k-k	25	680
878	893	528388	ACGATGCTTCTCCGCA	e-e-e-d ₍₁₀₎ -k-k-k	39	681
879	894	528389	CACGATGCTTCTCCGC	e-e-e-d ₍₁₀₎ -k-k-k	46	682
880	895	528390	TCACGATGCTTCTCCG	e-e-e-d ₍₁₀₎ -k-k-k	17	683
881	896	528391	CTCACGATGCTTCTCC	e-e-e-d ₍₁₀₎ -k-k-k	20	684
882	897	528392	ACTCACGATGCTTCTC	e-e-e-d ₍₁₀₎ -k-k-k	16	685
883	898	528393	CACTCACGATGCTTCT	e-e-e-d ₍₁₀₎ -k-k-k	39	686
885	900	528394	CTCACTCACGATGCTT	e-e-e-d ₍₁₀₎ -k-k-k	45	687
886	901	528395	GCTCACTCACGATGCT	e-e-e-d ₍₁₀₎ -k-k-k	37	688
888	903	528396	CAGCTCACTCACGATG	e-e-e-d ₍₁₀₎ -k-k-k	24	689
889	904	528397	CCAGCTCACTCACGAT	e-e-e-d ₍₁₀₎ -k-k-k	25	690
890	905	528398	GCCAGCTCACTCACGA	e-e-e-d ₍₁₀₎ -k-k-k	18	691
891	906	528399	CGCCAGCTCACTCACG	e-e-e-d ₍₁₀₎ -k-k-k	4	692
1068	1083	528477	AATTTGTTGACGGGTC	e-e-e-d ₍₁₀₎ -k-k-k	37	693
1069	1084	528478	TAATTTGTTGACGGGT	e-e-e-d ₍₁₀₎ -k-k-k	35	694
1070	1085	528479	TTAATTTGTTGACGGG	e-e-e-d ₍₁₀₎ -k-k-k	40	695
1072	1087	528480	TCTTAATTTGTTGACG	e-e-e-d ₍₁₀₎ -k-k-k	6	696
1087	1102	528481	GCAACTCCTCCAGTTT	e-e-e-d ₍₁₀₎ -k-k-k	42	697
1088	1103	528482	TGCAACTCCTCCAGTT	e-e-e-d ₍₁₀₎ -k-k-k	28	698
1094	1109	528483	TTTTGCTGCAACTCCT	e-e-e-d ₍₁₀₎ -k-k-k	49	699
1095	1110	528484	TTTTTGCTGCAACTCC	e-e-e-d ₍₁₀₎ -k-k-k	58	700
1114	1129	528485	GGTCCCCTTTGTAGGA	e-e-e-d ₍₁₀₎ -k-k-k	35	701
1115	1130	528486	GGGTCCCCTTTGTAGG	e-e-e-d ₍₁₀₎ -k-k-k	31	702
1129	1144	528487	GGTGCTGTACAATGGG	e-e-e-d ₍₁₀₎ -k-k-k	61	703
1130	1145	528488	CGGTGCTGTACAATGG	e-e-e-d ₍₁₀₎ -k-k-k	61	704
1131	1146	528489	CCGGTGCTGTACAATG	e-e-e-d ₍₁₀₎ -k-k-k	37	705
1132	1147	528490	GCCGGTGCTGTACAAT	e-e-e-d ₍₁₀₎ -k-k-k	33	706
1133	1148	528491	GGCCGGTGCTGTACAA	e-e-e-d ₍₁₀₎ -k-k-k	39	707
1134	1149	528492	CGGCCGGTGCTGTACA	e-e-e-d ₍₁₀₎ -k-k-k	38	708

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1136	1151	528493	ATCGGCCGGTGCTGTA	e-e-e-d ₍₁₀₎ -k-k-k	29	709
1137	1152	528494	CATCGGCCGGTGCTGT	e-e-e-d ₍₁₀₎ -k-k-k	43	710
1138	1153	528495	GCATCGGCCGGTGCTG	e-e-e-d ₍₁₀₎ -k-k-k	41	711
1139	1154	528496	AGCATCGGCCGGTGCT	e-e-e-d ₍₁₀₎ -k-k-k	18	712
1140	1155	528497	CAGCATCGGCCGGTGC	e-e-e-d ₍₁₀₎ -k-k-k	15	713
1141	1156	528498	CCAGCATCGGCCGGTG	e-e-e-d ₍₁₀₎ -k-k-k	39	714
1142	1157	528499	TCCAGCATCGGCCGGT	e-e-e-d ₍₁₀₎ -k-k-k	50	715
1144	1159	528500	CCTCCAGCATCGGCCG	e-e-e-d ₍₁₀₎ -k-k-k	58	716
1146	1161	528501	CTCCTCCAGCATCGGC	e-e-e-d ₍₁₀₎ -k-k-k	67	717
1147	1162	528502	TCTCCTCCAGCATCGG	e-e-e-d ₍₁₀₎ -k-k-k	76	718
1153	1168	528503	CGATTCTCTCCTCCAG	e-e-e-d ₍₁₀₎ -k-k-k	68	719
1154	1169	528504	ACGATTCTCTCCTCCA	e-e-e-d ₍₁₀₎ -k-k-k	69	720
1155	1170	528505	CACGATTCTCTCCTCC	e-e-e-d ₍₁₀₎ -k-k-k	68	721
1156	1171	528506	CCACGATTCTCTCCTC	e-e-e-d ₍₁₀₎ -k-k-k	45	722
1157	1172	528507	TCCACGATTCTCTCCT	e-e-e-d ₍₁₀₎ -k-k-k	42	723
1158	1173	528508	CTCCACGATTCTCTCC	e-e-e-d ₍₁₀₎ -k-k-k	41	724
1159	1174	528509	GCTCCACGATTCTCTC	e-e-e-d ₍₁₀₎ -k-k-k	32	725
1160	1175	528510	AGTCCACGATTCTCT	e-e-e-d ₍₁₀₎ -k-k-k	7	726
1161	1176	528511	CAGTCCACGATTCTC	e-e-e-d ₍₁₀₎ -k-k-k	5	727
1162	1177	528512	ACAGTCCACGATTCT	e-e-e-d ₍₁₀₎ -k-k-k	0	728
1163	1178	528513	AACAGTCCACGATTC	e-e-e-d ₍₁₀₎ -k-k-k	8	729
1184	1199	528514	GCACTTTTCATTAAGT	e-e-e-d ₍₁₀₎ -k-k-k	14	730
1185	1200	528515	GGCACTTTTCATTAAG	e-e-e-d ₍₁₀₎ -k-k-k	15	731
1199	1214	528516	CGTCCACCACAAAGG	e-e-e-d ₍₁₀₎ -k-k-k	46	732
1205	1220	528517	GGTGCCGCTCCACCA	e-e-e-d ₍₁₀₎ -k-k-k	55	733
1206	1221	528518	GGGCTGCCGCTCCACC	e-e-e-d ₍₁₀₎ -k-k-k	80	734
1207	1222	528519	AGGGCTGCCGCTCCAC	e-e-e-d ₍₁₀₎ -k-k-k	61	735
1208	1223	528520	CAGGGCTGCCGCTCCA	e-e-e-d ₍₁₀₎ -k-k-k	63	736
1211	1226	528521	ATGCAGGGCTGCCGCT	e-e-e-d ₍₁₀₎ -k-k-k	37	737
1212	1227	528522	CATGCAGGGCTGCCGC	e-e-e-d ₍₁₀₎ -k-k-k	38	738
1221	1236	528523	ATGCATGGGCATGCAG	e-e-e-d ₍₁₀₎ -k-k-k	26	739
1222	1237	528524	GATGCATGGGCATGCA	e-e-e-d ₍₁₀₎ -k-k-k	42	740
1223	1238	528525	GGATGCATGGGCATGC	e-e-e-d ₍₁₀₎ -k-k-k	43	741
1252	1267	528526	CGCCGGTCTTGATGAC	e-e-e-d ₍₁₀₎ -k-k-k	11	742
1253	1268	528527	ACGCCGGTCTTGATGA	e-e-e-d ₍₁₀₎ -k-k-k	0	743
1265	1280	528528	GTAGTGAAGTGGACGC	e-e-e-d ₍₁₀₎ -k-k-k	10	744
1284	1299	528529	GACCAGCAACCTGACT	e-e-e-d ₍₁₀₎ -k-k-k	22	745
1285	1300	528530	TGACCAGCAACCTGAC	e-e-e-d ₍₁₀₎ -k-k-k	31	746
1288	1303	528531	ATTTGACCAGCAACCT	e-e-e-d ₍₁₀₎ -k-k-k	48	747
1289	1304	528532	AATTTGACCAGCAACC	e-e-e-d ₍₁₀₎ -k-k-k	22	748
1290	1305	528533	GAATTTGACCAGCAAC	e-e-e-d ₍₁₀₎ -k-k-k	11	749
1293	1308	528534	AGGGAATTTGACCAGC	e-e-e-d ₍₁₀₎ -k-k-k	67	750

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1294	1309	528535	CAGGGAATTTGACCAG	e-e-e-d ₍₁₀₎ -k-k-k	50	751
1295	1310	528536	TCAGGGAATTTGACCA	e-e-e-d ₍₁₀₎ -k-k-k	38	752
1296	1311	528537	CTCAGGGAATTTGACC	e-e-e-d ₍₁₀₎ -k-k-k	17	753
1336	1351	528539	CTTTGTCAATGCACAC	e-e-e-d ₍₁₀₎ -k-k-k	67	754
1338	1353	528540	GTCTTTGTCAATGCAC	e-e-e-d ₍₁₀₎ -k-k-k	61	755
1339	1354	528541	AGTCTTTGTCAATGCA	e-e-e-d ₍₁₀₎ -k-k-k	65	756
1343	1358	528542	CCAGAGTCTTTGTCAA	e-e-e-d ₍₁₀₎ -k-k-k	10	757
1345	1360	528543	CCCCAGAGTCTTTGTC	e-e-e-d ₍₁₀₎ -k-k-k	7	758
1371	1386	528544	CCGGGATCCTCTGAGA	e-e-e-d ₍₁₀₎ -k-k-k	12	759
1372	1387	528545	TCCGGGATCCTCTGAG	e-e-e-d ₍₁₀₎ -k-k-k	11	760
1373	1388	528546	TTCCGGGATCCTCTGA	e-e-e-d ₍₁₀₎ -k-k-k	7	761
1374	1389	528547	TTTCCGGGATCCTCTG	e-e-e-d ₍₁₀₎ -k-k-k	14	762
1375	1390	528548	ATTTCGGGATCCTCT	e-e-e-d ₍₁₀₎ -k-k-k	14	763
1376	1391	528549	AATTTCGGGATCCTC	e-e-e-d ₍₁₀₎ -k-k-k	19	764
1377	1392	528550	AAATTTCCGGGATCCT	e-e-e-d ₍₁₀₎ -k-k-k	14	765
1379	1394	528551	TTAAATTTCCGGGATC	e-e-e-d ₍₁₀₎ -k-k-k	1	766
1380	1395	528552	GTTAAATTTCCGGGAT	e-e-e-d ₍₁₀₎ -k-k-k	9	767
1381	1396	528553	TGTAAATTTCCGGGA	e-e-e-d ₍₁₀₎ -k-k-k	0	768
1382	1397	528554	ATGTAAATTTCCGGG	e-e-e-d ₍₁₀₎ -k-k-k	12	769
1384	1399	528555	GAATGTAAATTTCCG	e-e-e-d ₍₁₀₎ -k-k-k	13	770
1392	1407	528556	TGTGCCCAGAATGTTA	e-e-e-d ₍₁₀₎ -k-k-k	18	771
1435	1450	528557	GGCTGCCGTTGTTGGA	e-e-e-d ₍₁₀₎ -k-k-k	48	772
1436	1451	528558	AGGCTGCCGTTGTTGG	e-e-e-d ₍₁₀₎ -k-k-k	38	773
1437	1452	528559	GAGGCTGCCGTTGTTG	e-e-e-d ₍₁₀₎ -k-k-k	24	98
1438	1453	528560	AGAGGCTGCCGTTGTT	e-e-e-d ₍₁₀₎ -k-k-k	27	774
1439	1454	528561	GAGAGGCTGCCGTTGT	e-e-e-d ₍₁₀₎ -k-k-k	10	775
1440	1455	528562	AGAGAGGCTGCCGTTG	e-e-e-d ₍₁₀₎ -k-k-k	17	776
1441	1456	528563	CAGAGAGGCTGCCGTT	e-e-e-d ₍₁₀₎ -k-k-k	27	777
1461	1476	528564	GGTCAAGTGTGTTGAAT	e-e-e-d ₍₁₀₎ -k-k-k	7	778
1471	1486	528565	GCTCCCTCAGGGTCAA	e-e-e-d ₍₁₀₎ -k-k-k	48	779
1496	1511	528566	GCTCGGCCCCCATTC	e-e-e-d ₍₁₀₎ -k-k-k	42	780
1497	1512	528567	GGCTCGGCCCCCATTC	e-e-e-d ₍₁₀₎ -k-k-k	45	781
1498	1513	528568	TGGCTCGGCCCCCAT	e-e-e-d ₍₁₀₎ -k-k-k	34	782
1499	1514	528569	TTGGCTCGGCCCCCAT	e-e-e-d ₍₁₀₎ -k-k-k	49	783
1517	1532	528570	ATCAGGGAAGCATCAC	e-e-e-d ₍₁₀₎ -k-k-k	22	104
1519	1534	528571	CAATCAGGGAAGCATC	e-e-e-d ₍₁₀₎ -k-k-k	13	784
1523	1538	528572	GTCACAATCAGGGAAG	e-e-e-d ₍₁₀₎ -k-k-k	30	785
1525	1540	528573	CAGTCACAATCAGGGA	e-e-e-d ₍₁₀₎ -k-k-k	27	786
1526	1541	528574	TCAGTCACAATCAGGG	e-e-e-d ₍₁₀₎ -k-k-k	51	787
1529	1544	528575	TCCTCAGTCACAATCA	e-e-e-d ₍₁₀₎ -k-k-k	14	788
1537	1552	528576	GGTGCAGCTCCTCAGT	e-e-e-d ₍₁₀₎ -k-k-k	28	789
1543	1558	528577	TGATCAGGTGCAGCTC	e-e-e-d ₍₁₀₎ -k-k-k	30	790

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1544	1559	528578	GTGATCAGGTGCAGCT	e-e-e-d ₍₁₀₎ -k-k-k	36	791
1545	1560	528579	GGTGATCAGGTGCAGC	e-e-e-d ₍₁₀₎ -k-k-k	39	792
1576	1591	528580	TGAGGCCTTGGTGATA	e-e-e-d ₍₁₀₎ -k-k-k	10	793
1578	1593	528581	CTTGAGGCCTTGGTGA	e-e-e-d ₍₁₀₎ -k-k-k	5	794
1579	1594	528582	TCTTGAGGCCTTGGTG	e-e-e-d ₍₁₀₎ -k-k-k	15	110
1580	1595	528583	ATCTTGAGGCCTTGGT	e-e-e-d ₍₁₀₎ -k-k-k	5	795
1581	1596	528584	AATCTTGAGGCCTTGG	e-e-e-d ₍₁₀₎ -k-k-k	15	796
1582	1597	528585	CAATCTTGAGGCCTTG	e-e-e-d ₍₁₀₎ -k-k-k	7	797
1583	1598	528586	TCAATCTTGAGGCCTT	e-e-e-d ₍₁₀₎ -k-k-k	9	798
1584	1599	528587	GTCAATCTTGAGGCCT	e-e-e-d ₍₁₀₎ -k-k-k	25	799
1585	1600	528588	GGTCAATCTTGAGGCC	e-e-e-d ₍₁₀₎ -k-k-k	26	800
1586	1601	528589	AGGTCAATCTTGAGGC	e-e-e-d ₍₁₀₎ -k-k-k	31	801
1587	1602	528590	TAGGTCAATCTTGAGG	e-e-e-d ₍₁₀₎ -k-k-k	27	802
1588	1603	528591	CTAGGTCAATCTTGAG	e-e-e-d ₍₁₀₎ -k-k-k	24	803
1590	1605	528592	CTCTAGGTCAATCTTG	e-e-e-d ₍₁₀₎ -k-k-k	33	804
1592	1607	528593	GTCTCTAGGTCAATCT	e-e-e-d ₍₁₀₎ -k-k-k	30	805
1594	1609	528594	GGGTCTCTAGGTCAAT	e-e-e-d ₍₁₀₎ -k-k-k	25	806
1595	1610	528595	TGGGTCTCTAGGTCAA	e-e-e-d ₍₁₀₎ -k-k-k	28	807
1596	1611	528596	GTGGGTCTCTAGGTCA	e-e-e-d ₍₁₀₎ -k-k-k	34	808
1597	1612	528597	AGTGGGTCTCTAGGTC	e-e-e-d ₍₁₀₎ -k-k-k	19	809
1599	1614	528598	GGAGTGGGTCTCTAGG	e-e-e-d ₍₁₀₎ -k-k-k	31	114
1600	1615	528599	AGGAGTGGGTCTCTAG	e-e-e-d ₍₁₀₎ -k-k-k	10	810
1601	1616	528600	AAGGAGTGGGTCTCTA	e-e-e-d ₍₁₀₎ -k-k-k	14	811
1602	1617	528601	CAAGGAGTGGGTCTCT	e-e-e-d ₍₁₀₎ -k-k-k	11	812
1609	1624	528602	CAACTGGCAAGGAGTG	e-e-e-d ₍₁₀₎ -k-k-k	17	813
1629	1644	528603	ACAGATGTTGGAGATC	e-e-e-d ₍₁₀₎ -k-k-k	8	814
1632	1647	528604	CTGACAGATGTTGGAG	e-e-e-d ₍₁₀₎ -k-k-k	11	815
1633	1648	528605	TCTGACAGATGTTGGA	e-e-e-d ₍₁₀₎ -k-k-k	25	119
1650	1665	528606	CGCCCAGGCATTTGGC	e-e-e-d ₍₁₀₎ -k-k-k	18	816
1651	1666	528607	ACGCCAGGCATTTGG	e-e-e-d ₍₁₀₎ -k-k-k	36	817
1677	1692	528608	GGTCAGCATGTTGTAC	e-e-e-d ₍₁₀₎ -k-k-k	11	818
1678	1693	528609	TGGTCAGCATGTTGTA	e-e-e-d ₍₁₀₎ -k-k-k	9	819
1680	1695	528610	GTTGGTCAGCATGTTG	e-e-e-d ₍₁₀₎ -k-k-k	19	820
1682	1697	528611	TTGTTGGTCAGCATGT	e-e-e-d ₍₁₀₎ -k-k-k	27	821
1711	1726	528612	GCTTGGTAAAAAAGTT	e-e-e-d ₍₁₀₎ -k-k-k	0	822
1712	1727	528613	GGCTTGGTAAAAAAGT	e-e-e-d ₍₁₀₎ -k-k-k	0	823
1713	1728	528614	GGGCTTGGTAAAAAAG	e-e-e-d ₍₁₀₎ -k-k-k	0	824
1736	1751	528615	ACTTGATCCCAGGTTTC	e-e-e-d ₍₁₀₎ -k-k-k	26	825
1741	1756	528616	CGGCCACTTGATCCCA	e-e-e-d ₍₁₀₎ -k-k-k	41	826
1742	1757	528617	TCGGCCACTTGATCCC	e-e-e-d ₍₁₀₎ -k-k-k	40	827
1743	1758	528618	CTCGGCCACTTGATCC	e-e-e-d ₍₁₀₎ -k-k-k	27	828
1744	1759	528619	CCTCGGCCACTTGATC	e-e-e-d ₍₁₀₎ -k-k-k	10	829

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1745	1760	528620	ACCTCGGCCACTTGAT	e-e-e-d ₍₁₀₎ -k-k-k	16	830
1746	1761	528621	GACCTCGGCCACTTGA	e-e-e-d ₍₁₀₎ -k-k-k	31	831
1747	1762	528622	GGACCTCGGCCACTTG	e-e-e-d ₍₁₀₎ -k-k-k	59	832
1748	1763	528623	AGGACCTCGGCCACTT	e-e-e-d ₍₁₀₎ -k-k-k	49	833
1749	1764	528624	CAGGACCTCGGCCACT	e-e-e-d ₍₁₀₎ -k-k-k	32	834
1753	1768	528625	AGCTCAGGACCTCGGC	e-e-e-d ₍₁₀₎ -k-k-k	28	835
1754	1769	528626	CAGCTCAGGACCTCGG	e-e-e-d ₍₁₀₎ -k-k-k	58	836
1755	1770	528627	CCAGCTCAGGACCTCG	e-e-e-d ₍₁₀₎ -k-k-k	56	837
1778	1793	528628	CGCTTGGTGGTGGAGG	e-e-e-d ₍₁₀₎ -k-k-k	15	838
1779	1794	528629	TCGCTTGGTGGTGGAG	e-e-e-d ₍₁₀₎ -k-k-k	9	839
1780	1795	528630	CTCGCTTGGTGGTGGGA	e-e-e-d ₍₁₀₎ -k-k-k	14	127
1781	1796	528631	CCTCGCTTGGTGGTGG	e-e-e-d ₍₁₀₎ -k-k-k	26	840
1782	1797	528632	TCCTCGCTTGGTGGTG	e-e-e-d ₍₁₀₎ -k-k-k	24	841
1783	1798	528633	GTCCTCGCTTGGTGGT	e-e-e-d ₍₁₀₎ -k-k-k	40	842
1784	1799	528634	AGTCCTCGCTTGGTGG	e-e-e-d ₍₁₀₎ -k-k-k	38	843
1785	1800	528635	CAGTCCTCGCTTGGTG	e-e-e-d ₍₁₀₎ -k-k-k	20	844
1786	1801	528636	TCAGTCCTCGCTTGGT	e-e-e-d ₍₁₀₎ -k-k-k	23	845
1787	1802	528637	CTCAGTCCTCGCTTGG	e-e-e-d ₍₁₀₎ -k-k-k	33	846
1788	1803	528638	GCTCAGTCCTCGCTTG	e-e-e-d ₍₁₀₎ -k-k-k	15	847
1789	1804	528639	TGCTCAGTCCTCGCTT	e-e-e-d ₍₁₀₎ -k-k-k	15	848
1791	1806	528640	GATGCTCAGTCCTCGC	e-e-e-d ₍₁₀₎ -k-k-k	43	849
1792	1807	528641	CGATGCTCAGTCCTCG	e-e-e-d ₍₁₀₎ -k-k-k	46	850
1793	1808	528642	TCGATGCTCAGTCCTC	e-e-e-d ₍₁₀₎ -k-k-k	39	851
1794	1809	528643	CTCGATGCTCAGTCCT	e-e-e-d ₍₁₀₎ -k-k-k	32	852
1795	1810	528644	GCTCGATGCTCAGTCC	e-e-e-d ₍₁₀₎ -k-k-k	43	129
1796	1811	528645	TGCTCGATGCTCAGTC	e-e-e-d ₍₁₀₎ -k-k-k	22	853
1797	1812	528646	CTGCTCGATGCTCAGT	e-e-e-d ₍₁₀₎ -k-k-k	38	854
1799	1814	528647	AGCTGCTCGATGCTCA	e-e-e-d ₍₁₀₎ -k-k-k	40	855
1800	1815	528648	CAGCTGCTCGATGCTC	e-e-e-d ₍₁₀₎ -k-k-k	39	856
1802	1817	528649	GTCAGCTGCTCGATGC	e-e-e-d ₍₁₀₎ -k-k-k	32	857
1803	1818	528650	AGTCAGCTGCTCGATG	e-e-e-d ₍₁₀₎ -k-k-k	10	858
1804	1819	528651	TAGTCAGCTGCTCGAT	e-e-e-d ₍₁₀₎ -k-k-k	4	859
1805	1820	528652	GTAGTCAGCTGCTCGA	e-e-e-d ₍₁₀₎ -k-k-k	17	860
1806	1821	528653	TGTAGTCAGCTGCTCG	e-e-e-d ₍₁₀₎ -k-k-k	28	861
1807	1822	528654	GTGTAGTCAGCTGCTC	e-e-e-d ₍₁₀₎ -k-k-k	31	862
1808	1823	528655	AGTGTAGTCAGCTGCT	e-e-e-d ₍₁₀₎ -k-k-k	30	863
1809	1824	528656	CAGTGTAGTCAGCTGC	e-e-e-d ₍₁₀₎ -k-k-k	30	864
1810	1825	528657	CCAGTGTAGTCAGCTG	e-e-e-d ₍₁₀₎ -k-k-k	23	865
1811	1826	528658	GCCAGTGTAGTCAGCT	e-e-e-d ₍₁₀₎ -k-k-k	30	866
1832	1847	528659	CCAGGTCCCAAGAGTT	e-e-e-d ₍₁₀₎ -k-k-k	12	867
1852	1867	528660	GACACCCTGAATAATT	e-e-e-d ₍₁₀₎ -k-k-k	10	868
1853	1868	528661	TGACACCCTGAATAAT	e-e-e-d ₍₁₀₎ -k-k-k	10	869

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1856	1871	528662	ATCTGACACCCTGAAT	e-e-e-d ₍₁₀₎ -k-k-k	12	870
1857	1872	528663	GATCTGACACCCTGAA	e-e-e-d ₍₁₀₎ -k-k-k	22	871
1859	1874	528664	GTGATCTGACACCCTG	e-e-e-d ₍₁₀₎ -k-k-k	61	872
1861	1876	528665	ATGTGATCTGACACCC	e-e-e-d ₍₁₀₎ -k-k-k	36	873
1865	1880	528666	GCCCATGTGATCTGAC	e-e-e-d ₍₁₀₎ -k-k-k	46	874
1866	1881	528667	AGCCCATGTGATCTGA	e-e-e-d ₍₁₀₎ -k-k-k	36	137
1867	1882	528668	TAGCCCATGTGATCTG	e-e-e-d ₍₁₀₎ -k-k-k	44	875
1869	1884	528669	TTTAGCCCATGTGATC	e-e-e-d ₍₁₀₎ -k-k-k	12	876
1907	1922	528670	AAGGAGAAGCCCTTGC	e-e-e-d ₍₁₀₎ -k-k-k	35	877
1925	1940	528671	TTGTCCAGCCAGACCC	e-e-e-d ₍₁₀₎ -k-k-k	40	878
1926	1941	528672	ATTGTCCAGCCAGACC	e-e-e-d ₍₁₀₎ -k-k-k	36	879
1927	1942	528673	TATTGTCCAGCCAGAC	e-e-e-d ₍₁₀₎ -k-k-k	23	880
1928	1943	528674	ATATTGTCCAGCCAGA	e-e-e-d ₍₁₀₎ -k-k-k	24	881
1929	1944	528675	GATATTGTCCAGCCAG	e-e-e-d ₍₁₀₎ -k-k-k	52	882
1931	1946	528676	ATGATATTGTCCAGCC	e-e-e-d ₍₁₀₎ -k-k-k	41	883
1933	1948	528677	CAATGATATTGTCCAG	e-e-e-d ₍₁₀₎ -k-k-k	23	884
1935	1950	528678	GTCAATGATATTGTCC	e-e-e-d ₍₁₀₎ -k-k-k	32	885
1936	1951	528679	GGTCAATGATATTGTC	e-e-e-d ₍₁₀₎ -k-k-k	26	886
1941	1956	528680	CACAAGGTCAATGATA	e-e-e-d ₍₁₀₎ -k-k-k	5	887
1942	1957	528681	TCACAAGGTCAATGAT	e-e-e-d ₍₁₀₎ -k-k-k	9	888
1948	1963	518340	ACTTTTTCACAAGGTC	e-e-e-d ₍₁₀₎ -k-k-k	52	153
1950	1965	528682	GTACTTTTTCACAAGG	e-e-e-d ₍₁₀₎ -k-k-k	21	889
1954	1969	528683	GGATGTACTTTTTCAC	e-e-e-d ₍₁₀₎ -k-k-k	0	890
1958	1973	528684	GCCAGGATGTACTTTT	e-e-e-d ₍₁₀₎ -k-k-k	0	891
1962	1977	528685	AAGGGCCAGGATGTAC	e-e-e-d ₍₁₀₎ -k-k-k	0	892
1963	1978	528686	AAAGGGCCAGGATGTA	e-e-e-d ₍₁₀₎ -k-k-k	0	893
2004	2019	528687	CCGCTCCTTACTGATA	e-e-e-d ₍₁₀₎ -k-k-k	21	894
2010	2025	528688	CCGCTCCCGCTCCTTA	e-e-e-d ₍₁₀₎ -k-k-k	32	895
2014	2029	528689	TGGCCCGCTCCCGCTC	e-e-e-d ₍₁₀₎ -k-k-k	52	896
2015	2030	528690	ATGGCCCGCTCCCGCT	e-e-e-d ₍₁₀₎ -k-k-k	41	897
2017	2032	528691	AGATGGCCCGCTCCCG	e-e-e-d ₍₁₀₎ -k-k-k	51	898
2018	2033	528692	AAGATGGCCCGCTCCC	e-e-e-d ₍₁₀₎ -k-k-k	45	899
2019	2034	528693	CAAGATGGCCCGCTCC	e-e-e-d ₍₁₀₎ -k-k-k	46	900
2020	2035	528694	TCAAGATGGCCCGCTC	e-e-e-d ₍₁₀₎ -k-k-k	27	901
2022	2037	528695	GCTCAAGATGGCCCGC	e-e-e-d ₍₁₀₎ -k-k-k	54	902
2023	2038	528696	TGCTCAAGATGGCCCG	e-e-e-d ₍₁₀₎ -k-k-k	46	903
2024	2039	528697	GTGCTCAAGATGGCCC	e-e-e-d ₍₁₀₎ -k-k-k	60	904
2041	2056	528698	AGGTGCCTGGAGGCTT	e-e-e-d ₍₁₀₎ -k-k-k	17	905
2093	2108	528699	CAAGTGAAAGTGACGC	e-e-e-d ₍₁₀₎ -k-k-k	2	161
2094	2109	528700	CCAAGTGAAAGTGACG	e-e-e-d ₍₁₀₎ -k-k-k	13	906
2095	2110	528701	CCCAAGTGAAAGTGAC	e-e-e-d ₍₁₀₎ -k-k-k	14	907
2128	2143	528702	GGATCTGGGTCTTACC	e-e-e-d ₍₁₀₎ -k-k-k	22	908

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2129	2144	528703	TGGATCTGGGTCTTAC	e-e-e-d ₍₁₀₎ -k-k-k	22	909
2131	2146	528704	ACTGGATCTGGGTCTT	e-e-e-d ₍₁₀₎ -k-k-k	21	165
2133	2148	528705	GGACTGGATCTGGGTC	e-e-e-d ₍₁₀₎ -k-k-k	38	910
2138	2153	528706	TCCACGGACTGGATCT	e-e-e-d ₍₁₀₎ -k-k-k	13	911
2139	2154	528707	TTCCACGGACTGGATC	e-e-e-d ₍₁₀₎ -k-k-k	19	912
2140	2155	528708	GTTCCACGGACTGGAT	e-e-e-d ₍₁₀₎ -k-k-k	2	913
2141	2156	528709	GGTTCCACGGACTGGA	e-e-e-d ₍₁₀₎ -k-k-k	42	914
2142	2157	528710	TGGTTCCACGGACTGG	e-e-e-d ₍₁₀₎ -k-k-k	63	915
2143	2158	528711	ATGGTTCCACGGACTG	e-e-e-d ₍₁₀₎ -k-k-k	62	916
2144	2159	528712	TATGGTTCCACGGACT	e-e-e-d ₍₁₀₎ -k-k-k	35	917
2146	2161	528713	TGTATGGTTCCACGGA	e-e-e-d ₍₁₀₎ -k-k-k	40	918
2147	2162	528714	GTGTATGGTTCCACGG	e-e-e-d ₍₁₀₎ -k-k-k	48	919
2193	2208	528715	GCCCATGATGATTTCA	e-e-e-d ₍₁₀₎ -k-k-k	36	920
2194	2209	528716	AGCCCATGATGATTTTC	e-e-e-d ₍₁₀₎ -k-k-k	25	921
2195	2210	528717	TAGCCCATGATGATTT	e-e-e-d ₍₁₀₎ -k-k-k	27	922
2196	2211	528718	ATAGCCCATGATGATT	e-e-e-d ₍₁₀₎ -k-k-k	19	923
2197	2212	528719	TATAGCCCATGATGAT	e-e-e-d ₍₁₀₎ -k-k-k	14	924
2198	2213	528720	TTATAGCCCATGATGA	e-e-e-d ₍₁₀₎ -k-k-k	14	925
2199	2214	528721	CTTATAGCCCATGATG	e-e-e-d ₍₁₀₎ -k-k-k	21	926
2200	2215	528722	TCTTATAGCCCATGAT	e-e-e-d ₍₁₀₎ -k-k-k	0	927
2201	2216	528723	ATCTTATAGCCCATGA	e-e-e-d ₍₁₀₎ -k-k-k	17	928
2202	2217	528724	GATCTTATAGCCCATG	e-e-e-d ₍₁₀₎ -k-k-k	35	929
2203	2218	528725	TGATCTTATAGCCCAT	e-e-e-d ₍₁₀₎ -k-k-k	45	930
2204	2219	528726	ATGATCTTATAGCCCA	e-e-e-d ₍₁₀₎ -k-k-k	67	931
2205	2220	528727	CATGATCTTATAGCCC	e-e-e-d ₍₁₀₎ -k-k-k	45	932
2206	2221	528728	CCATGATCTTATAGCC	e-e-e-d ₍₁₀₎ -k-k-k	38	175
2207	2222	528729	TCCATGATCTTATAGC	e-e-e-d ₍₁₀₎ -k-k-k	0	933
2208	2223	528730	ATCCATGATCTTATAG	e-e-e-d ₍₁₀₎ -k-k-k	12	934
2213	2228	528731	GTAGCATCCATGATCT	e-e-e-d ₍₁₀₎ -k-k-k	14	935
2214	2229	528732	GGTAGCATCCATGATC	e-e-e-d ₍₁₀₎ -k-k-k	25	936
2217	2232	528733	ATTGGTAGCATCCATG	e-e-e-d ₍₁₀₎ -k-k-k	22	937
2218	2233	528734	TATTGGTAGCATCCAT	e-e-e-d ₍₁₀₎ -k-k-k	15	938
2219	2234	528735	ATATTGGTAGCATCCA	e-e-e-d ₍₁₀₎ -k-k-k	28	939
2264	2279	528736	TCCTTGGGAATGTCAG	e-e-e-d ₍₁₀₎ -k-k-k	30	940
2266	2281	528737	CCTCCTTGGGAATGTC	e-e-e-d ₍₁₀₎ -k-k-k	30	181
2275	2290	528738	CGAATGCCTCCTCCTT	e-e-e-d ₍₁₀₎ -k-k-k	29	186
2277	2292	528739	TCCGAATGCCTCCTCC	e-e-e-d ₍₁₀₎ -k-k-k	33	941
2278	2293	528740	TTCCGAATGCCTCCTC	e-e-e-d ₍₁₀₎ -k-k-k	27	942
2279	2294	528741	TTTCCGAATGCCTCCT	e-e-e-d ₍₁₀₎ -k-k-k	20	943
2280	2295	528742	CTTTCCGAATGCCTCC	e-e-e-d ₍₁₀₎ -k-k-k	25	944
2281	2296	528743	ACTTTCCGAATGCCTC	e-e-e-d ₍₁₀₎ -k-k-k	39	945
2283	2298	528744	ATACTTTCCGAATGCC	e-e-e-d ₍₁₀₎ -k-k-k	44	946

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2285	2300	528745	CAATACTTTCCGAATG	e-e-e-d ₍₁₀₎ -k-k-k	0	947
2286	2301	528746	ACAATACTTTCCGAAT	e-e-e-d ₍₁₀₎ -k-k-k	0	948
2288	2303	528747	CGACAATACTTTCCGA	e-e-e-d ₍₁₀₎ -k-k-k	11	949
2289	2304	528748	CCGACAATACTTTCCG	e-e-e-d ₍₁₀₎ -k-k-k	31	950
2290	2305	528749	GCCGACAATACTTTCC	e-e-e-d ₍₁₀₎ -k-k-k	18	951
2291	2306	528750	GGCCGACAATACTTTC	e-e-e-d ₍₁₀₎ -k-k-k	16	952
2293	2308	528751	CTGGCCGACAATACTT	e-e-e-d ₍₁₀₎ -k-k-k	18	953
2294	2309	528752	TCTGGCCGACAATACT	e-e-e-d ₍₁₀₎ -k-k-k	8	954
2295	2310	528753	CTCTGGCCGACAATAC	e-e-e-d ₍₁₀₎ -k-k-k	0	955
2296	2311	528754	TCTCTGGCCGACAATA	e-e-e-d ₍₁₀₎ -k-k-k	6	188
2297	2312	528755	CTCTCTGGCCGACAAT	e-e-e-d ₍₁₀₎ -k-k-k	18	956
2298	2313	528756	GCTCTCTGGCCGACAA	e-e-e-d ₍₁₀₎ -k-k-k	35	957
2299	2314	528757	GGCTCTCTGGCCGACA	e-e-e-d ₍₁₀₎ -k-k-k	57	958
2300	2315	528758	TGGCTCTCTGGCCGAC	e-e-e-d ₍₁₀₎ -k-k-k	64	959
2301	2316	528759	CTGGCTCTCTGGCCGA	e-e-e-d ₍₁₀₎ -k-k-k	12	960
2326	2341	528760	TACCTGGGTCAGCTTC	e-e-e-d ₍₁₀₎ -k-k-k	21	961
2328	2343	528761	GCTACCTGGGTCAGCT	e-e-e-d ₍₁₀₎ -k-k-k	18	962
2329	2344	528762	CGCTACCTGGGTCAGC	e-e-e-d ₍₁₀₎ -k-k-k	28	963
2330	2345	528763	GCGCTACCTGGGTCAG	e-e-e-d ₍₁₀₎ -k-k-k	26	964
2349	2364	528764	GGTCTTCAGGTATGGG	e-e-e-d ₍₁₀₎ -k-k-k	38	965
2350	2365	528765	TGGTCTTCAGGTATGG	e-e-e-d ₍₁₀₎ -k-k-k	12	966
2352	2367	528766	CTTGGTCTTCAGGTAT	e-e-e-d ₍₁₀₎ -k-k-k	0	967
2353	2368	528767	ACTTGGTCTTCAGGTA	e-e-e-d ₍₁₀₎ -k-k-k	10	190
2358	2373	528768	GATAAACTTGGTCTTC	e-e-e-d ₍₁₀₎ -k-k-k	9	968
2360	2375	528769	CAGATAAACTTGGTCT	e-e-e-d ₍₁₀₎ -k-k-k	15	969
2361	2376	528770	ACAGATAAACTTGGTC	e-e-e-d ₍₁₀₎ -k-k-k	7	970
2369	2384	528771	GGTGTACACAGATAAA	e-e-e-d ₍₁₀₎ -k-k-k	35	971
2373	2388	528772	CGTTGGTGTACACAG	e-e-e-d ₍₁₀₎ -k-k-k	52	972
2387	2402	528773	GTATTGCTGCAGGTCG	e-e-e-d ₍₁₀₎ -k-k-k	49	194
2388	2403	528774	GGTATTGCTGCAGGTC	e-e-e-d ₍₁₀₎ -k-k-k	48	973
2389	2404	528775	TGGTATTGCTGCAGGT	e-e-e-d ₍₁₀₎ -k-k-k	35	974
2390	2405	528776	ATGGTATTGCTGCAGG	e-e-e-d ₍₁₀₎ -k-k-k	20	975
2392	2407	528777	CAATGGTATTGCTGCA	e-e-e-d ₍₁₀₎ -k-k-k	24	976
2393	2408	528778	TCAATGGTATTGCTGC	e-e-e-d ₍₁₀₎ -k-k-k	15	977
2394	2409	528779	GTCAATGGTATTGCTG	e-e-e-d ₍₁₀₎ -k-k-k	16	978
2395	2410	528780	GGTCAATGGTATTGCT	e-e-e-d ₍₁₀₎ -k-k-k	34	196
2396	2411	528781	AGGTCAATGGTATTGC	e-e-e-d ₍₁₀₎ -k-k-k	26	979
2397	2412	528782	CAGGTCAATGGTATTG	e-e-e-d ₍₁₀₎ -k-k-k	16	980
2398	2413	528783	GCAGGTCAATGGTATT	e-e-e-d ₍₁₀₎ -k-k-k	10	981
2399	2414	528784	GGCAGGTCAATGGTAT	e-e-e-d ₍₁₀₎ -k-k-k	32	982
2400	2415	528785	CGGCAGGTCAATGGTA	e-e-e-d ₍₁₀₎ -k-k-k	39	983
2401	2416	528786	TCGGCAGGTCAATGGT	e-e-e-d ₍₁₀₎ -k-k-k	51	984

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2403	2418	528787	CATCGGCAGGTCAATG	e-e-e-d ₍₁₀₎ -k-k-k	26	198
2404	2419	528788	ACATCGGCAGGTCAAT	e-e-e-d ₍₁₀₎ -k-k-k	20	985
2405	2420	528789	GACATCGGCAGGTCAA	e-e-e-d ₍₁₀₎ -k-k-k	42	986
2406	2421	528790	GGACATCGGCAGGTCA	e-e-e-d ₍₁₀₎ -k-k-k	58	987
2407	2422	528791	GGGACATCGGCAGGTC	e-e-e-d ₍₁₀₎ -k-k-k	68	988
2423	2438	528792	GAATCTAAAGTGCGGG	e-e-e-d ₍₁₀₎ -k-k-k	46	200
2424	2439	528793	TGAATCTAAAGTGCGG	e-e-e-d ₍₁₀₎ -k-k-k	43	989
2427	2442	528794	CAATGAATCTAAAGTG	e-e-e-d ₍₁₀₎ -k-k-k	20	990
2462	2477	528795	GGTTCAGCACCTTCAC	e-e-e-d ₍₁₀₎ -k-k-k	13	991
2463	2478	528796	GGGTTTCAGCACCTTCA	e-e-e-d ₍₁₀₎ -k-k-k	24	992
2464	2479	528797	AGGGTTCAGCACCTTC	e-e-e-d ₍₁₀₎ -k-k-k	23	993
2465	2480	528798	GAGGGTTCAGCACCTT	e-e-e-d ₍₁₀₎ -k-k-k	18	994
2466	2481	528799	TGAGGGTTCAGCACCT	e-e-e-d ₍₁₀₎ -k-k-k	24	995
2490	2505	528800	GAGGGACTCAAAGTGC	e-e-e-d ₍₁₀₎ -k-k-k	28	996
2492	2507	528801	GTGAGGGACTCAAAGT	e-e-e-d ₍₁₀₎ -k-k-k	22	997
2493	2508	528802	GGTGAGGGACTCAAAC	e-e-e-d ₍₁₀₎ -k-k-k	20	998
2494	2509	528803	AGGTGAGGGACTCAAA	e-e-e-d ₍₁₀₎ -k-k-k	13	999
2495	2510	528804	AAGGTGAGGGACTCAA	e-e-e-d ₍₁₀₎ -k-k-k	20	1000
2497	2512	528805	CAAAGGTGAGGGACTC	e-e-e-d ₍₁₀₎ -k-k-k	20	1001
2498	2513	528806	TCAAAGGTGAGGGACT	e-e-e-d ₍₁₀₎ -k-k-k	18	1002
2506	2521	528807	ACTCCATGTCAAAGGT	e-e-e-d ₍₁₀₎ -k-k-k	54	1003
2510	2525	528808	GTCAACTCCATGTCAA	e-e-e-d ₍₁₀₎ -k-k-k	39	1004
2511	2526	528809	GGTCAACTCCATGTCA	e-e-e-d ₍₁₀₎ -k-k-k	56	1005
2513	2528	528810	GAGGTCAACTCCATGT	e-e-e-d ₍₁₀₎ -k-k-k	41	1006
2514	2529	528811	CGAGGTCAACTCCATG	e-e-e-d ₍₁₀₎ -k-k-k	45	1007
2515	2530	528812	CCGAGGTCAACTCCAT	e-e-e-d ₍₁₀₎ -k-k-k	45	1008
2517	2532	528813	CTCCGAGGTCAACTCC	e-e-e-d ₍₁₀₎ -k-k-k	58	1009
2518	2533	528814	ACTCCGAGGTCAACTC	e-e-e-d ₍₁₀₎ -k-k-k	40	1010
2519	2534	528815	CACTCCGAGGTCAACT	e-e-e-d ₍₁₀₎ -k-k-k	30	1011
2551	2566	528816	CGTTCTCAGCTCCTCA	e-e-e-d ₍₁₀₎ -k-k-k	54	1012
2554	2569	528817	TTCCGTTCTCAGCTCC	e-e-e-d ₍₁₀₎ -k-k-k	53	1013
2555	2570	528818	CTTCCGTTCTCAGCTC	e-e-e-d ₍₁₀₎ -k-k-k	27	1014
2556	2571	528819	GCTTCCGTTCTCAGCT	e-e-e-d ₍₁₀₎ -k-k-k	35	1015
2557	2572	528820	AGCTTCCGTTCTCAGC	e-e-e-d ₍₁₀₎ -k-k-k	38	1016
2558	2573	528821	CAGCTTCCGTTCTCAG	e-e-e-d ₍₁₀₎ -k-k-k	53	1017
2559	2574	528822	GCAGCTTCCGTTCTCA	e-e-e-d ₍₁₀₎ -k-k-k	66	1018
2614	2629	528823	TTGGCTGTGTGAGGG	e-e-e-d ₍₁₀₎ -k-k-k	62	1019
2615	2630	528824	GTTTGGCTGTGTGAGG	e-e-e-d ₍₁₀₎ -k-k-k	50	1020
2616	2631	528825	GGTTTGGCTGTGTGAG	e-e-e-d ₍₁₀₎ -k-k-k	15	1021
2641	2656	528826	AAGTTAGTAGTTTCAG	e-e-e-d ₍₁₀₎ -k-k-k	20	1022
2677	2692	528827	GCAGAAAGTAGGAGATT	e-e-e-d ₍₁₀₎ -k-k-k	28	1023
2690	2705	528828	TTGCTCAAAGATAGCA	e-e-e-d ₍₁₀₎ -k-k-k	39	1024

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2691	2706	528829	ATTGCTCAAAGATAGC	e-e-e-d ₍₁₀₎ -k-k-k	37	1025
2692	2707	528830	GATTGCTCAAAGATAG	e-e-e-d ₍₁₀₎ -k-k-k	22	1026
2694	2709	528831	CAGATTGCTCAAAGAT	e-e-e-d ₍₁₀₎ -k-k-k	26	1027
2695	2710	528832	CCAGATTGCTCAAAGA	e-e-e-d ₍₁₀₎ -k-k-k	41	1028
2699	2714	528833	GTGCCCAGATTGCTCA	e-e-e-d ₍₁₀₎ -k-k-k	77	1029
2738	2753	528834	GCAGATCACCCACATT	e-e-e-d ₍₁₀₎ -k-k-k	49	1030
2743	2758	528835	TAAAAGCAGATCACCC	e-e-e-d ₍₁₀₎ -k-k-k	40	1031
2809	2824	528836	CTAGCCACCCCCCGCC	e-e-e-d ₍₁₀₎ -k-k-k	19	1032
2810	2825	528837	TCTAGCCACCCCCCGC	e-e-e-d ₍₁₀₎ -k-k-k	9	1033
2811	2826	528838	CTCTAGCCACCCCCCG	e-e-e-d ₍₁₀₎ -k-k-k	16	1034
2908	2923	528839	GGAGGCACTTGTCTAA	e-e-e-d ₍₁₀₎ -k-k-k	56	235
2909	2924	528840	AGGAGGCACTTGTCTA	e-e-e-d ₍₁₀₎ -k-k-k	62	1036
2910	2925	528841	CAGGAGGCACTTGTCT	e-e-e-d ₍₁₀₎ -k-k-k	52	1037
2911	2926	528842	CCAGGAGGCACTTGTCT	e-e-e-d ₍₁₀₎ -k-k-k	59	1038
2932	2947	528843	GGCAGAAGGATGCCCGC	e-e-e-d ₍₁₀₎ -k-k-k	35	1039
2945	2960	528844	GCTTACAGAAACAGGC	e-e-e-d ₍₁₀₎ -k-k-k	62	1040
2980	2995	528845	CAGGAGTATGTAGCTA	e-e-e-d ₍₁₀₎ -k-k-k	65	1041
2981	2996	528846	CCAGGAGTATGTAGCT	e-e-e-d ₍₁₀₎ -k-k-k	80	1042
2982	2997	528847	GCCAGGAGTATGTAGC	e-e-e-d ₍₁₀₎ -k-k-k	72	1043
2983	2998	528848	TGCCAGGAGTATGTAG	e-e-e-d ₍₁₀₎ -k-k-k	46	1044
2984	2999	528849	ATGCCAGGAGTATGTA	e-e-e-d ₍₁₀₎ -k-k-k	59	241
3001	3016	528850	CAAGGTAAAAAGTGC	e-e-e-d ₍₁₀₎ -k-k-k	10	243
3008	3023	528851	ATGTCAGCAAGGTAA	e-e-e-d ₍₁₀₎ -k-k-k	61	1045
3010	3025	528852	GGATGTCAGCAAGGTT	e-e-e-d ₍₁₀₎ -k-k-k	88	1046
3012	3027	528853	TTGGATGTCAGCAAGG	e-e-e-d ₍₁₀₎ -k-k-k	91	1047
3016	3031	518349	CTATTTGGATGTCAGC	e-e-e-d ₍₁₀₎ -k-k-k	85	245
3030	3045	528854	GATAGTCCTATCTTCT	e-e-e-d ₍₁₀₎ -k-k-k	42	1048
3091	3106	528855	ACAGTGTTTTTTGCC	e-e-e-d ₍₁₀₎ -k-k-k	59	1049
3108	3123	528856	AGAAAGGCTATGCTGA	e-e-e-d ₍₁₀₎ -k-k-k	56	1050
3452	3467	528857	GAGGCTGTAACTGAA	e-e-e-d ₍₁₀₎ -k-k-k	40	1051
3458	3473	528858	ACCAAGGAGGCTGTAA	e-e-e-d ₍₁₀₎ -k-k-k	26	1052
3474	3489	528859	GCTGAATGCTTAAAGC	e-e-e-d ₍₁₀₎ -k-k-k	36	1053
4022	4037	518344	GCCACTGGATATCACC	e-e-e-d ₍₁₀₎ -k-k-k	55	317

Example 12: Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

Gapmers from the study described in Example 11, above, exhibiting significant *in vitro* inhibition of STAT3 were tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 23.4375 nM, 93.75 nM, 375.0 nM, and 1,500.0 nM concentrations of antisense oligonucleotide, as specified in Table 12. After a treatment period of approximately 16 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR.

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Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 12 and was calculated by plotting the concentrations of oligonucleotides used versus the percent inhibition of STAT3 mRNA expression achieved at each concentration, and noting the concentration of oligonucleotide at which 50% inhibition of STAT3 mRNA expression was achieved compared to the control. As illustrated in Table 12, STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 12
Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

ISIS No	23.4375 nM	93.75 nM	375.0 nM	1500.0 nM	IC ₅₀ (μ M)
518340	0	8	28	63	1.0
518349	13	30	68	90	0.2
528189	8	13	43	71	0.5
528204	4	24	53	79	0.3
528205	0	9	59	80	0.4
528208	0	19	56	84	0.3
528209	0	28	58	90	0.3
528210	0	16	49	87	0.3
528211	0	10	47	86	0.4
528212	0	16	42	83	0.4
528214	0	25	55	88	0.3
528215	3	16	53	82	0.3
528237	13	19	33	73	0.6
528245	3	16	53	78	0.4
528263	0	3	32	76	0.6
528264	9	0	19	50	>1.5
528268	0	7	25	63	1.0
528269	0	11	39	77	0.5
528270	5	9	48	79	0.4
528271	0	14	37	81	0.5
528327	0	0	26	72	0.8
528347	0	2	25	69	0.9
528357	0	17	36	69	0.6
528389	0	3	19	82	0.7
528501	0	17	40	69	0.6
528502	0	10	35	76	0.6

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528503	3	1	38	70	0.7
528504	0	19	45	72	0.5
528505	0	7	41	73	0.6
528518	0	24	51	81	0.3
528534	0	8	32	72	0.7
528539	0	7	39	73	0.6
528557	0	9	26	53	>1.5
528565	4	12	31	57	1.3
528567	8	13	25	54	>1.5
528569	9	19	37	60	0.8
528574	5	17	32	62	0.9
528622	10	4	29	68	0.9
528623	0	13	24	62	1.1
528626	1	0	34	68	0.8
528627	22	19	30	64	1.0
528664	0	14	37	74	0.5
528675	0	10	28	62	1.0
528689	0	16	33	65	0.7
528691	0	3	34	61	0.9
528695	1	4	36	66	0.8
528697	3	15	39	72	0.5
528710	13	16	28	63	1.0
528711	8	13	14	62	>1.5
528726	0	8	36	72	0.6
528757	4	10	29	76	0.6
528758	1	5	28	62	1.1
528772	0	2	21	63	1.2
528773	9	8	28	70	0.8
528791	4	9	41	69	0.6
528822	0	0	40	46	>1.5
528833	0	23	47	82	0.4
528846	10	19	49	85	0.3
528847	0	19	45	75	0.4
528852	5	33	66	93	0.2
528853	19	46	77	95	0.1

Example 13: Antisense inhibition of human STAT3 in HuVEC cells

Antisense oligonucleotides were designed targeting a human STAT3 nucleic acid and were tested for their effect on human STAT3 mRNA expression *in vitro*. The chimeric antisense oligonucleotides in Tables 13 and 14 are gapmers 16 or 17 nucleotides in length having various chemical modifications. Each gapmer comprises a central gap segment consisting of nine or ten 2'-deoxynucleosides and is flanked on both sides

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(in the 5' and 3' directions) by wings comprising 1, 2, 3, 4, or 5 nucleotides each. Each of the nucleotides in the wings comprise a 2'-MOE sugar modification or a cEt sugar modification. Gapmer motifs include 3-10-3, 4-9-3, 2-10-4, 1-10-5, and 3-10-4. The chemistry column of Tables 13 and 14 provides the sugar motif of each gapmer, wherein 'e' indicates a 2'-MOE nucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'd' indicates a 2'- deoxynucleoside. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5'-methylcytosines.

Potency of the chimeric antisense oligonucleotides was compared to ISIS 481464, ISIS 518344, and ISIS 518349 (described previously herein).

Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

"Human Target start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Human Target stop site" indicates the 3'-most nucleoside to which the gapmer is targeted in the human gene sequence. Each gapmer listed in Table 13 is targeted to human STAT3 mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_139276.2). Each gapmer listed in Table 14 is targeted to human STAT3 genomic sequence, designated herein as SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_010755.14 truncated from nucleotides 4185000 to 4264000).

Table 13

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

Human Start Site	Human Stop Site	ISIS No	Sequence	Chemistry	% inhibition	SEQ ID NO
728	743	530423	AGATTCTCTACCACTT	k-d(10)-k-e-k-e-e	70	1054
729	745	530053	GGAGATTCTCTACCACT	e-e-k-d(10)-k-e-k-e	84	1055
729	744	530373	GAGATTCTCTACCACT	e-k-d(10)-k-e-k-e	85	1056
730	745	530121	GGAGATTCTCTACCAC	e-k-k-d(10)-k-k-e	77	53
730	745	530168	GGAGATTCTCTACCAC	e-e-k-d(10)-k-k-e	75	53
730	745	530218	GGAGATTCTCTACCAC	e-d-k-d(10)-k-k-e	61	53
730	745	530268	GGAGATTCTCTACCAC	e-d-d-k-d(9)-k-k-e	76	53
730	745	530318	GGAGATTCTCTACCAC	e-e-e-e-d(9)-k-k-e	27	53
786	801	530424	ATCTTGCATGTCTCCT	k-d(10)-k-e-k-e-e	42	1057
787	803	530058	AGATCTTGCATGTCTCC	e-e-k-d(10)-k-e-k-e	73	1058
787	802	530374	GATCTTGCATGTCTCC	e-k-d(10)-k-e-k-e	71	647

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788	803	530122	AGATCTTGCATGTCTC	e-k-k-d(10)-k-k-e	80	57
788	803	530169	AGATCTTGCATGTCTC	e-e-k-d(10)-k-k-e	72	57
788	803	530219	AGATCTTGCATGTCTC	e-d-k-d(10)-k-k-e	55	57
788	803	530269	AGATCTTGCATGTCTC	e-d-d-k-d(9)-k-k-e	76	57
788	803	530319	AGATCTTGCATGTCTC	e-e-e-e-d(9)-k-k-e	30	57
892	907	528400	CCGCCAGCTCACTCAC	e-e-e-d(10)-k-k-k	57	66
893	908	528401	CCCGCCAGCTCACTCA	e-e-e-d(10)-k-k-k	57	1059
894	909	528402	CCCCGCCAGCTCACTC	e-e-e-d(10)-k-k-k	42	1060
897	912	528403	AAGCCCCGCCAGCTCA	e-e-e-d(10)-k-k-k	72	1061
898	913	528404	AAAGCCCCGCCAGCTC	e-e-e-d(10)-k-k-k	52	1062
899	914	528405	AAAAGCCCCGCCAGCT	e-e-e-d(10)-k-k-k	27	1063
900	915	528406	CAAAAGCCCCGCCAGC	e-e-e-d(10)-k-k-k	29	1064
901	916	528407	ACAAAAGCCCCGCCAG	e-e-e-d(10)-k-k-k	9	1065
903	918	528408	TGACAAAAGCCCCGCC	e-e-e-d(10)-k-k-k	10	1066
904	919	528409	CTGACAAAAGCCCCGC	e-e-e-d(10)-k-k-k	31	1067
905	920	528410	GCTGACAAAAGCCCCG	e-e-e-d(10)-k-k-k	39	1068
906	921	528411	CGCTGACAAAAGCCCC	e-e-e-d(10)-k-k-k	49	1069
907	922	528412	TCGCTGACAAAAGCCC	e-e-e-d(10)-k-k-k	39	1070
908	923	528413	ATCGCTGACAAAAGCC	e-e-e-d(10)-k-k-k	20	1071
909	924	528414	CATCGCTGACAAAAGC	e-e-e-d(10)-k-k-k	10	1072
911	926	528415	TCCATCGCTGACAAA	e-e-e-d(10)-k-k-k	11	1073
912	927	528416	CTCCATCGCTGACAAA	e-e-e-d(10)-k-k-k	15	1074
913	928	528417	ACTCCATCGCTGACAA	e-e-e-d(10)-k-k-k	22	1075
914	929	528418	TACTCCATCGCTGACA	e-e-e-d(10)-k-k-k	19	1076
915	930	528419	GTACTCCATCGCTGAC	e-e-e-d(10)-k-k-k	37	1077
916	931	528420	CGTACTCCATCGCTGA	e-e-e-d(10)-k-k-k	35	1078
930	945	528421	GAGAGTTTTCTGCACG	e-e-e-d(10)-k-k-k	36	1079
932	947	528422	GTGAGAGTTTTCTGCA	e-e-e-d(10)-k-k-k	22	1080
951	966	528423	GTCAGCCAGCTCCTCG	e-e-e-d(10)-k-k-k	49	1081
962	977	528424	CGCCTCTTCCAGTCAG	e-e-e-d(10)-k-k-k	42	1082
964	979	528425	GCCGCCTCTTCCAGTC	e-e-e-d(10)-k-k-k	44	1083
965	980	528426	TGCCGCCTCTTCCAGT	e-e-e-d(10)-k-k-k	15	1084
970	985	528427	TCTGTTGCCGCCTCTT	e-e-e-d(10)-k-k-k	9	1085
971	986	528428	ATCTGTTGCCGCCTCT	e-e-e-d(10)-k-k-k	30	1086
972	987	528429	AATCTGTTGCCGCCTC	e-e-e-d(10)-k-k-k	23	1087
973	988	528430	CAATCTGTTGCCGCCT	e-e-e-d(10)-k-k-k	12	1088
974	989	528431	GCAATCTGTTGCCGCC	e-e-e-d(10)-k-k-k	48	1089
975	990	528432	GGCAATCTGTTGCCGC	e-e-e-d(10)-k-k-k	18	1090
976	991	528433	AGGCAATCTGTTGCCG	e-e-e-d(10)-k-k-k	0	1091
977	992	528434	CAGGCAATCTGTTGCC	e-e-e-d(10)-k-k-k	8	1092
978	993	528435	GCAGGCAATCTGTTGC	e-e-e-d(10)-k-k-k	13	1093
982	997	528436	CAATGCAGGCAATCTG	e-e-e-d(10)-k-k-k	9	1094

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983	998	528437	CCAATGCAGGCAATCT	e-e-e-d(10)-k-k-k	26	1095
984	999	528438	TCCAATGCAGGCAATC	e-e-e-d(10)-k-k-k	10	1096
985	1000	528439	CTCCAATGCAGGCAAT	e-e-e-d(10)-k-k-k	2	1097
986	1001	528440	CCTCCAATGCAGGCAA	e-e-e-d(10)-k-k-k	28	1098
1003	1018	528441	GGCAGATGTTGGGCGG	e-e-e-d(10)-k-k-k	8	1099
1004	1019	528442	AGGCAGATGTTGGGCG	e-e-e-d(10)-k-k-k	0	1100
1005	1020	528443	TAGGCAGATGTTGGGC	e-e-e-d(10)-k-k-k	1	1101
1006	1021	528444	CTAGGCAGATGTTGGG	e-e-e-d(10)-k-k-k	0	1102
1007	1022	528445	TCTAGGCAGATGTTGG	e-e-e-d(10)-k-k-k	7	1103
1008	1023	528446	ATCTAGGCAGATGTTG	e-e-e-d(10)-k-k-k	3	1104
1010	1025	528447	CGATCTAGGCAGATGT	e-e-e-d(10)-k-k-k	9	72
1011	1026	528448	CCGATCTAGGCAGATG	e-e-e-d(10)-k-k-k	13	1105
1013	1028	528449	AGCCGATCTAGGCAGA	e-e-e-d(10)-k-k-k	4	1106
1014	1029	528450	TAGCCGATCTAGGCAG	e-e-e-d(10)-k-k-k	11	1107
1015	1030	528451	CTAGCCGATCTAGGCA	e-e-e-d(10)-k-k-k	5	1108
1016	1031	528452	TCTAGCCGATCTAGGC	e-e-e-d(10)-k-k-k	5	1109
1017	1032	528453	TTCTAGCCGATCTAGG	e-e-e-d(10)-k-k-k	24	1110
1018	1033	528454	TTTCTAGCCGATCTAG	e-e-e-d(10)-k-k-k	29	1111
1019	1034	528455	TTTTCTAGCCGATCTA	e-e-e-d(10)-k-k-k	28	1112
1020	1035	528456	GTTTTCTAGCCGATCT	e-e-e-d(10)-k-k-k	42	1113
1022	1037	528457	CAGTTTTCTAGCCGAT	e-e-e-d(10)-k-k-k	50	1114
1023	1038	528458	CCAGTTTTCTAGCCGA	e-e-e-d(10)-k-k-k	70	1115
1024	1039	528459	TCCAGTTTTCTAGCCG	e-e-e-d(10)-k-k-k	56	1116
1025	1040	528460	ATCCAGTTTTCTAGCC	e-e-e-d(10)-k-k-k	42	1117
1029	1044	528461	CGTTATCCAGTTTTCT	e-e-e-d(10)-k-k-k	47	1118
1043	1058	528462	GATTCTGCTAATGACG	e-e-e-d(10)-k-k-k	42	1119
1044	1059	528463	AGATTCTGCTAATGAC	e-e-e-d(10)-k-k-k	38	1120
1048	1063	528464	GTTGAGATTCTGCTAA	e-e-e-d(10)-k-k-k	30	1121
1049	1064	528465	AGTTGAGATTCTGCTA	e-e-e-d(10)-k-k-k	48	1122
1056	1071	528466	GGTCTGAAGTTGAGAT	e-e-e-d(10)-k-k-k	27	1123
1058	1073	528467	CGGGTCTGAAGTTGAG	e-e-e-d(10)-k-k-k	44	1124
1059	1074	528468	ACGGGTCTGAAGTTGA	e-e-e-d(10)-k-k-k	41	1125
1060	1075	528469	GACGGGTCTGAAGTTG	e-e-e-d(10)-k-k-k	45	1126
1061	1076	528470	TGACGGGTCTGAAGTT	e-e-e-d(10)-k-k-k	34	1127
1062	1077	528471	TTGACGGGTCTGAAGT	e-e-e-d(10)-k-k-k	19	1128
1063	1078	528472	GTTGACGGGTCTGAAG	e-e-e-d(10)-k-k-k	21	1129
1064	1079	528473	TGTTGACGGGTCTGAA	e-e-e-d(10)-k-k-k	37	1130
1065	1080	528474	TTGTTGACGGGTCTGA	e-e-e-d(10)-k-k-k	55	1131
1066	1081	528475	TTTGTTGACGGGTCTG	e-e-e-d(10)-k-k-k	63	1132
1067	1082	528476	ATTTGTTGACGGGTCT	e-e-e-d(10)-k-k-k	65	1133
1899	1914	530425	GCCCTTGCCAGCCATG	k-d(10)-k-e-k-e-e	73	1134
1900	1916	530054	AAGCCCTTGCCAGCCAT	e-e-k-d(10)-k-e-k-e	75	1135

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1900	1915	530375	AGCCCTTGCCAGCCAT	e-k-d(10)-k-e-k-e	77	1136
1901	1916	530123	AAGCCCTTGCCAGCCA	e-k-k-d(10)-k-k-e	86	144
1901	1916	530170	AAGCCCTTGCCAGCCA	e-e-k-d(10)-k-k-e	87	144
1901	1916	530220	AAGCCCTTGCCAGCCA	e-d-k-d(10)-k-k-e	74	144
1901	1916	530270	AAGCCCTTGCCAGCCA	e-d-d-k-d(9)-k-k-e	87	144
1901	1916	530320	AAGCCCTTGCCAGCCA	e-e-e-d(9)-k-k-e	17	144
1946	1961	530426	TTTTTCACAAGGTCAA	k-d(10)-k-e-k-e-e	55	1137
1947	1963	530059	ACTTTTTCACAAGGTCA	e-e-k-d(10)-k-e-k-e	73	1138
1947	1962	530376	CTTTTTCACAAGGTCA	e-k-d(10)-k-e-k-e	77	1139
1948	1963	530124	ACTTTTTCACAAGGTC	e-k-k-d(10)-k-k-e	79	153
1948	1963	530171	ACTTTTTCACAAGGTC	e-e-k-d(10)-k-k-e	69	153
1948	1963	530221	ACTTTTTCACAAGGTC	e-d-k-d(10)-k-k-e	64	153
1948	1963	530271	ACTTTTTCACAAGGTC	e-d-d-k-d(9)-k-k-e	73	153
1948	1963	530321	ACTTTTTCACAAGGTC	e-e-e-d(9)-k-k-e	44	153
2204	2219	530427	ATGATCTTATAGCCCA	k-d(10)-k-e-k-e-e	43	931
2205	2221	530060	CCATGATCTTATAGCCC	e-e-k-d(10)-k-e-k-e	77	1140
2205	2220	530377	CATGATCTTATAGCCC	e-k-d(10)-k-e-k-e	66	932
2206	2221	530125	CCATGATCTTATAGCC	e-k-k-d(10)-k-k-e	65	175
2206	2221	530172	CCATGATCTTATAGCC	e-e-k-d(10)-k-k-e	59	175
2206	2221	530222	CCATGATCTTATAGCC	e-d-k-d(10)-k-k-e	48	175
2206	2221	530272	CCATGATCTTATAGCC	e-d-d-k-d(9)-k-k-e	63	175
2206	2221	530322	CCATGATCTTATAGCC	e-e-e-d(9)-k-k-e	55	175
2679	2694	530428	TAGCAGAAGTAGGAGA	k-d(10)-k-e-k-e-e	49	1141
2680	2696	530061	GATAGCAGAAGTAGGAG	e-e-k-d(10)-k-e-k-e	49	1142
2680	2695	530378	ATAGCAGAAGTAGGAG	e-k-d(10)-k-e-k-e	48	1143
2681	2696	530126	GATAGCAGAAGTAGGA	e-k-k-d(10)-k-k-e	70	223
2681	2696	530173	GATAGCAGAAGTAGGA	e-e-k-d(10)-k-k-e	62	223
2681	2696	530223	GATAGCAGAAGTAGGA	e-d-k-d(10)-k-k-e	44	223
2681	2696	530273	GATAGCAGAAGTAGGA	e-d-d-k-d(9)-k-k-e	63	223
2681	2696	530323	GATAGCAGAAGTAGGA	e-e-e-d(9)-k-k-e	63	223
3012	3027	530513	TTGGATGTCAGCAAGG	k-d(10)-k-e-k-e-e	88	1047
3013	3028	530507	TTTGGATGTCAGCAAG	e-k-d(10)-k-e-k-e	86	1144
3013	3028	530514	TTTGGATGTCAGCAAG	k-d(10)-k-e-k-e-e	80	1144
3014	3029	530430	ATTTGGATGTCAGCAA	k-d(10)-k-e-k-e-e	87	1145
3014	3029	530468	ATTTGGATGTCAGCAA	e-k-k-d(10)-k-k-e	81	1145
3014	3029	530476	ATTTGGATGTCAGCAA	e-e-k-d(10)-k-k-e	82	1145
3014	3029	530484	ATTTGGATGTCAGCAA	e-d-k-d(10)-k-k-e	74	1145
3014	3029	530492	ATTTGGATGTCAGCAA	e-d-d-k-d(9)-k-k-e	83	1145
3014	3029	530500	ATTTGGATGTCAGCAA	e-e-e-d(9)-k-k-e	56	1145
3014	3029	530508	ATTTGGATGTCAGCAA	e-k-d(10)-k-e-k-e	83	1145
3015	3031	530062	CTATTTGGATGTCAGCA	e-e-k-d(10)-k-e-k-e	94	1146
3015	3030	530380	TATTTGGATGTCAGCA	e-k-d(10)-k-e-k-e	94	1147

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3015	3030	530469	TATTTGGATGTCAGCA	e-k-k-d(10)-k-k-e	91	1147
3015	3030	530477	TATTTGGATGTCAGCA	e-e-k-d(10)-k-k-e	87	1147
3015	3030	530485	TATTTGGATGTCAGCA	e-d-k-d(10)-k-k-e	87	1147
3015	3030	530493	TATTTGGATGTCAGCA	e-d-d-k-d(9)-k-k-e	81	1147
3015	3030	530501	TATTTGGATGTCAGCA	e-e-e-e-d(9)-k-k-e	74	1147
3015	3030	530515	TATTTGGATGTCAGCA	k-d(10)-k-e-k-e-e	87	1147
3016	3031	481464	CTATTTGGATGTCAGC	k-k-k-d(10)-k-k-k	93	245
3016	3031	518349	CTATTTGGATGTCAGC	e-e-e-d(10)-k-k-k	58	245
3016	3031	519637	CTATTTGGATGTCAGC	e-k-k-d(10)-k-k-e	96	245
3016	3031	530175	CTATTTGGATGTCAGC	e-e-k-d(10)-k-k-e	93	245
3016	3031	530225	CTATTTGGATGTCAGC	e-d-k-d(10)-k-k-e	85	245
3016	3031	530275	CTATTTGGATGTCAGC	e-d-d-k-d(9)-k-k-e	91	245
3016	3031	530325	CTATTTGGATGTCAGC	e-e-e-e-d(9)-k-k-e	91	245
3017	3032	530470	TCTATTTGGATGTCAG	e-k-k-d(10)-k-k-e	91	1148
3017	3032	530478	TCTATTTGGATGTCAG	e-e-k-d(10)-k-k-e	87	1148
3017	3032	530486	TCTATTTGGATGTCAG	e-d-k-d(10)-k-k-e	84	1148
3017	3032	530494	TCTATTTGGATGTCAG	e-d-d-k-d(9)-k-k-e	60	1148
3017	3032	530502	TCTATTTGGATGTCAG	e-e-e-e-d(9)-k-k-e	64	1148
3017	3032	530509	TCTATTTGGATGTCAG	e-k-d(10)-k-e-k-e	80	1148
3018	3033	530471	TTCTATTTGGATGTCA	e-k-k-d(10)-k-k-e	83	1149
3018	3033	530479	TTCTATTTGGATGTCA	e-e-k-d(10)-k-k-e	74	1149
3018	3033	530487	TTCTATTTGGATGTCA	e-d-k-d(10)-k-k-e	71	1149
3018	3033	530495	TTCTATTTGGATGTCA	e-d-d-k-d(9)-k-k-e	68	1149
3018	3033	530503	TTCTATTTGGATGTCA	e-e-e-e-d(9)-k-k-e	53	1149
3459	3474	530431	CACCAAGGAGGCTGTT	k-d(10)-k-e-k-e-e	44	1150
3460	3476	530055	AGCACCAAGGAGGCTGT	e-e-k-d(10)-k-e-k-e	45	1151
3460	3475	530381	GCACCAAGGAGGCTGT	e-k-d(10)-k-e-k-e	74	1152
3461	3476	530128	AGCACCAAGGAGGCTG	e-k-k-d(10)-k-k-e	52	257
3461	3476	530176	AGCACCAAGGAGGCTG	e-e-k-d(10)-k-k-e	66	257
3461	3476	530226	AGCACCAAGGAGGCTG	e-d-k-d(10)-k-k-e	51	257
3461	3476	530276	AGCACCAAGGAGGCTG	e-d-d-k-d(9)-k-k-e	70	257
3461	3476	530326	AGCACCAAGGAGGCTG	e-e-e-e-d(9)-k-k-e	52	257
3527	3542	528860	GGTTTGACCTGAAGCC	e-e-e-d(10)-k-k-k	58	1153
3528	3543	528861	GGGTTTGACCTGAAGC	e-e-e-d(10)-k-k-k	42	1154
3529	3544	528862	AGGGTTTGACCTGAAG	e-e-e-d(10)-k-k-k	57	1155
3530	3545	528863	AAGGGTTTGACCTGAA	e-e-e-d(10)-k-k-k	43	1156
3531	3546	528864	TAAGGGTTTGACCTGA	e-e-e-d(10)-k-k-k	50	1157
3532	3547	528865	TTAAGGGTTTGACCTG	e-e-e-d(10)-k-k-k	32	1158
3547	3562	528866	GCAGCTTCAGATGTCT	e-e-e-d(10)-k-k-k	60	1159
3548	3563	528867	TGCAGCTTCAGATGTC	e-e-e-d(10)-k-k-k	47	1160
3583	3598	530388	CTTAAACCTTCCTATT	k-d(10)-k-e-k-e-e	14	1161
3584	3599	530338	CCTTAAACCTTCCTAT	e-k-d(10)-k-e-k-e	47	1162

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3585	3600	530086	TCCTTAAACCTTCCTA	e-k-k-d(10)-k-k-e	58	273
3585	3600	530133	TCCTTAAACCTTCCTA	e-e-k-d(10)-k-k-e	53	273
3585	3600	530183	TCCTTAAACCTTCCTA	e-d-k-d(10)-k-k-e	52	273
3585	3600	530233	TCCTTAAACCTTCCTA	e-d-d-k-d(9)-k-k-e	29	273
3585	3600	530283	TCCTTAAACCTTCCTA	e-e-e-e-d(9)-k-k-e	32	273
3590	3605	528868	GATTCTCCTTAAACCT	e-e-e-d(10)-k-k-k	45	1163
3591	3606	530389	AGATTCTCCTTAAACC	k-d(10)-k-e-k-e-e	44	1164
3592	3607	530339	TAGATTCTCCTTAAAC	e-k-d(10)-k-e-k-e	41	1165
3593	3608	530087	TTAGATTCTCCTTAAA	e-k-k-d(10)-k-k-e	43	1166
3593	3608	530134	TTAGATTCTCCTTAAA	e-e-k-d(10)-k-k-e	28	1166
3593	3608	530184	TTAGATTCTCCTTAAA	e-d-k-d(10)-k-k-e	13	1166
3593	3608	530234	TTAGATTCTCCTTAAA	e-d-d-k-d(9)-k-k-e	15	1166
3593	3608	530284	TTAGATTCTCCTTAAA	e-e-e-e-d(9)-k-k-e	14	1166
3595	3610	530390	GCTTAGATTCTCCTTA	k-d(10)-k-e-k-e-e	83	1167
3596	3611	530340	TGCTTAGATTCTCCTT	e-k-d(10)-k-e-k-e	89	1168
3597	3612	528869	ATGCTTAGATTCTCCT	e-e-e-d(10)-k-k-k	83	1169
3597	3612	530088	ATGCTTAGATTCTCCT	e-k-k-d(10)-k-k-e	90	1169
3597	3612	530135	ATGCTTAGATTCTCCT	e-e-k-d(10)-k-k-e	91	1169
3597	3612	530185	ATGCTTAGATTCTCCT	e-d-k-d(10)-k-k-e	85	1169
3597	3612	530235	ATGCTTAGATTCTCCT	e-d-d-k-d(9)-k-k-e	28	1169
3597	3612	530285	ATGCTTAGATTCTCCT	e-e-e-e-d(9)-k-k-e	86	1169
3597	3612	530391	ATGCTTAGATTCTCCT	k-d(10)-k-e-k-e-e	79	1169
3598	3614	530021	AAATGCTTAGATTCTCC	e-e-k-d(10)-k-e-k-e	87	1170
3598	3613	530341	AATGCTTAGATTCTCC	e-k-d(10)-k-e-k-e	88	1171
3599	3614	530089	AAATGCTTAGATTCTC	e-k-k-d(10)-k-k-e	71	1172
3599	3614	530136	AAATGCTTAGATTCTC	e-e-k-d(10)-k-k-e	66	1172
3599	3614	530186	AAATGCTTAGATTCTC	e-d-k-d(10)-k-k-e	51	1172
3599	3614	530236	AAATGCTTAGATTCTC	e-d-d-k-d(9)-k-k-e	74	1172
3599	3614	530286	AAATGCTTAGATTCTC	e-e-e-e-d(9)-k-k-e	56	1172
3682	3697	528870	GTAAGCACCTCTGCC	e-e-e-d(10)-k-k-k	26	1173
3684	3699	528871	TTGTAAGCACCTCTG	e-e-e-d(10)-k-k-k	14	1174
3686	3701	528872	GGTTGTAAGCACCTC	e-e-e-d(10)-k-k-k	47	1175
3687	3702	528873	AGGTTGTAAGCACCT	e-e-e-d(10)-k-k-k	40	1176
3688	3703	528874	AAGGTTGTAAGCACCC	e-e-e-d(10)-k-k-k	54	1177
3690	3705	528875	TCAAGGTTGTAAGCAC	e-e-e-d(10)-k-k-k	15	1178
3691	3706	528876	GTCAAGGTTGTAAGCA	e-e-e-d(10)-k-k-k	28	1179
3692	3707	528877	AGTCAAGGTTGTAAGC	e-e-e-d(10)-k-k-k	28	1180
3694	3709	528878	GGAGTCAAGGTTGTAA	e-e-e-d(10)-k-k-k	6	1181
3695	3710	528879	GGGAGTCAAGGTTGTA	e-e-e-d(10)-k-k-k	22	1182
3714	3729	530392	GATCAAGTCCAGGGAG	k-d(10)-k-e-k-e-e	47	1183
3715	3731	530022	CAGATCAAGTCCAGGGA	e-e-k-d(10)-k-e-k-e	80	1184
3715	3730	530342	AGATCAAGTCCAGGGA	e-k-d(10)-k-e-k-e	70	1185

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3715	3730	530393	AGATCAAGTCCAGGGA	k-d(10)-k-e-k-e-e	46	1185
3716	3732	530023	GCAGATCAAGTCCAGGG	e-e-k-d(10)-k-e-k-e	74	1186
3716	3731	530090	CAGATCAAGTCCAGGG	e-k-k-d(10)-k-k-e	78	1187
3716	3731	530137	CAGATCAAGTCCAGGG	e-e-k-d(10)-k-k-e	76	1187
3716	3731	530187	CAGATCAAGTCCAGGG	e-d-k-d(10)-k-k-e	68	1187
3716	3731	530237	CAGATCAAGTCCAGGG	e-d-d-k-d(9)-k-k-e	36	1187
3716	3731	530287	CAGATCAAGTCCAGGG	e-e-e-e-d(9)-k-k-e	56	1187
3716	3731	530343	CAGATCAAGTCCAGGG	e-k-d(10)-k-e-k-e	68	1187
3716	3731	530394	CAGATCAAGTCCAGGG	k-d(10)-k-e-k-e-e	49	1187
3717	3732	518343	GCAGATCAAGTCCAGG	e-e-e-d(10)-k-k-k	5	1188
3717	3733	530024	AGCAGATCAAGTCCAGG	e-e-k-d(10)-k-e-k-e	79	1189
3717	3732	530091	GCAGATCAAGTCCAGG	e-k-k-d(10)-k-k-e	81	1188
3717	3732	530138	GCAGATCAAGTCCAGG	e-e-k-d(10)-k-k-e	81	1188
3717	3732	530188	GCAGATCAAGTCCAGG	e-d-k-d(10)-k-k-e	78	1188
3717	3732	530238	GCAGATCAAGTCCAGG	e-d-d-k-d(9)-k-k-e	29	1188
3717	3732	530288	GCAGATCAAGTCCAGG	e-e-e-e-d(9)-k-k-e	69	1188
3717	3732	530344	GCAGATCAAGTCCAGG	e-k-d(10)-k-e-k-e	85	1188
3718	3733	530092	AGCAGATCAAGTCCAG	e-k-k-d(10)-k-k-e	85	1190
3718	3733	530139	AGCAGATCAAGTCCAG	e-e-k-d(10)-k-k-e	79	1190
3718	3733	530189	AGCAGATCAAGTCCAG	e-d-k-d(10)-k-k-e	77	1190
3718	3733	530239	AGCAGATCAAGTCCAG	e-d-d-k-d(9)-k-k-e	61	1190
3718	3733	530289	AGCAGATCAAGTCCAG	e-e-e-e-d(9)-k-k-e	75	1190
3720	3735	528880	ACAGCAGATCAAGTCC	e-e-e-d(10)-k-k-k	65	1191
3721	3736	528881	AACAGCAGATCAAGTC	e-e-e-d(10)-k-k-k	44	1192
3737	3752	528882	ACAACCTAGCCTCTGA	e-e-e-d(10)-k-k-k	39	1193
3738	3753	528883	AACAACCTAGCCTCTG	e-e-e-d(10)-k-k-k	46	1194
3740	3755	528884	GAAACAACCTAGCCTC	e-e-e-d(10)-k-k-k	37	1195
3741	3756	528885	AGAAACAACCTAGCCT	e-e-e-d(10)-k-k-k	20	1196
3742	3757	528886	CAGAAACAACCTAGCC	e-e-e-d(10)-k-k-k	21	1197
3755	3770	528887	GATAAGGCACCCACAG	e-e-e-d(10)-k-k-k	25	1198
3756	3771	528888	TGATAAGGCACCCACA	e-e-e-d(10)-k-k-k	12	1199
3757	3772	528889	CTGATAAGGCACCCAC	e-e-e-d(10)-k-k-k	25	1200
3759	3774	528890	CCCTGATAAGGCACCC	e-e-e-d(10)-k-k-k	42	1201
3760	3775	528891	GCCCTGATAAGGCACC	e-e-e-d(10)-k-k-k	49	1202
3765	3780	528892	TCCCAGCCCTGATAAG	e-e-e-d(10)-k-k-k	0	1203
3767	3782	528893	TATCCCAGCCCTGATA	e-e-e-d(10)-k-k-k	0	1204
3770	3785	528894	AAGTATCCCAGCCCTG	e-e-e-d(10)-k-k-k	25	1205
3771	3786	528895	GAAGTATCCCAGCCCT	e-e-e-d(10)-k-k-k	39	1206
3772	3787	528896	AGAAGTATCCCAGCCC	e-e-e-d(10)-k-k-k	22	1207
3773	3788	528897	CAGAAGTATCCCAGCC	e-e-e-d(10)-k-k-k	36	1208
3892	3907	528898	TGAGACCAGGATTCTT	e-e-e-d(10)-k-k-k	41	1209
3896	3911	528899	GTCCTGAGACCAGGAT	e-e-e-d(10)-k-k-k	19	1210

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3977	3992	528900	AGCTCAACCAGACACG	e-e-e-d(10)-k-k-k	54	311
3979	3994	528901	TGAGCTCAACCAGACA	e-e-e-d(10)-k-k-k	40	1211
3984	3999	528902	TTCCCTGAGCTCAACC	e-e-e-d(10)-k-k-k	32	1212
3992	4007	528903	GAACCATATTCCCTGA	e-e-e-d(10)-k-k-k	30	313
3995	4010	528904	TAAGAACCATATTCCC	e-e-e-d(10)-k-k-k	27	1213
4022	4037	518344	GCCACTGGATATCACC	e-e-e-d(10)-k-k-k	89	317
4067	4082	528905	TAAGCCTTTGCCCTGC	e-e-e-d(10)-k-k-k	64	1214
4068	4083	528906	GTAAGCCTTTGCCCTG	e-e-e-d(10)-k-k-k	53	1215
4069	4084	528907	AGTAAGCCTTTGCCCT	e-e-e-d(10)-k-k-k	45	1216
4070	4085	528908	CAGTAAGCCTTTGCCC	e-e-e-d(10)-k-k-k	40	1217
4072	4087	528909	ATCAGTAAGCCTTTGC	e-e-e-d(10)-k-k-k	53	1218
4073	4088	528910	TATCAGTAAGCCTTTG	e-e-e-d(10)-k-k-k	47	1219
4077	4092	528911	AGTTTATCAGTAAGCC	e-e-e-d(10)-k-k-k	58	1220
4083	4098	528912	GACTCAAGTTTATCAG	e-e-e-d(10)-k-k-k	37	1221
4085	4100	528913	CAGACTCAAGTTTATC	e-e-e-d(10)-k-k-k	39	1222
4086	4101	528914	GCAGACTCAAGTTTAT	e-e-e-d(10)-k-k-k	0	1223
4087	4102	528915	GGCAGACTCAAGTTTA	e-e-e-d(10)-k-k-k	1	1224
4088	4103	528916	GGGCAGACTCAAGTTT	e-e-e-d(10)-k-k-k	0	1225
4089	4104	528917	AGGGCAGACTCAAGTT	e-e-e-d(10)-k-k-k	9	1226
4091	4106	528918	CGAGGGCAGACTCAAG	e-e-e-d(10)-k-k-k	2	1227
4093	4108	528919	TACGAGGGCAGACTCA	e-e-e-d(10)-k-k-k	20	324
4094	4109	528920	ATACGAGGGCAGACTC	e-e-e-d(10)-k-k-k	14	1228
4095	4110	528921	CATACGAGGGCAGACT	e-e-e-d(10)-k-k-k	0	1229
4096	4111	528922	TCATACGAGGGCAGAC	e-e-e-d(10)-k-k-k	8	1230
4098	4113	528923	CCTCATACGAGGGCAG	e-e-e-d(10)-k-k-k	2	1231
4099	4114	528924	CCCTCATACGAGGGCA	e-e-e-d(10)-k-k-k	2	1232
4100	4115	528925	ACCCTCATACGAGGGC	e-e-e-d(10)-k-k-k	0	1233
4225	4240	528926	TACGCACAGGAGAGGC	e-e-e-d(10)-k-k-k	20	1233
4226	4241	528927	ATACGCACAGGAGAGG	e-e-e-d(10)-k-k-k	0	1234
4227	4242	528928	CATACGCACAGGAGAG	e-e-e-d(10)-k-k-k	6	1235
4228	4243	528929	CCATACGCACAGGAGA	e-e-e-d(10)-k-k-k	4	1236
4229	4244	528930	CCCATACGCACAGGAG	e-e-e-d(10)-k-k-k	36	1237
4230	4245	528931	TCCCATACGCACAGGA	e-e-e-d(10)-k-k-k	22	1238
4231	4246	528932	TTCCCATACGCACAGG	e-e-e-d(10)-k-k-k	32	1239
4232	4247	528933	GTTCCCATACGCACAG	e-e-e-d(10)-k-k-k	45	1240
4233	4248	528934	TGTTCCCATACGCACA	e-e-e-d(10)-k-k-k	36	1241
4234	4249	528935	GTGTTCCCATACGCAC	e-e-e-d(10)-k-k-k	20	1242
4234	4249	530395	GTGTTCCCATACGCAC	k-d(10)-k-e-k-e-e	71	1242
4235	4250	528936	GGTGTTCCTACGCA	e-e-e-d(10)-k-k-k	71	1243
4235	4251	530025	AGGTGTTCCTACGCA	e-e-k-d(10)-k-e-k-e	90	1244
4235	4250	530345	GGTGTTCCTACGCA	e-k-d(10)-k-e-k-e	93	1243
4235	4250	530396	GGTGTTCCTACGCA	k-d(10)-k-e-k-e-e	71	1243

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4236	4251	528937	AGGTGTTCCCATACGC	e-e-e-d(10)-k-k-k	73	1245
4236	4252	530026	TAGGTGTTCCCATACGC	e-e-k-d(10)-k-e-k-e	87	1246
4236	4251	530093	AGGTGTTCCCATACGC	e-k-k-d(10)-k-k-e	95	1245
4236	4251	530140	AGGTGTTCCCATACGC	e-e-k-d(10)-k-k-e	89	1245
4236	4251	530190	AGGTGTTCCCATACGC	e-d-k-d(10)-k-k-e	82	1245
4236	4251	530240	AGGTGTTCCCATACGC	e-d-d-k-d(9)-k-k-e	50	1245
4236	4251	530290	AGGTGTTCCCATACGC	e-e-e-e-d(9)-k-k-e	69	1245
4236	4251	530346	AGGTGTTCCCATACGC	e-k-d(10)-k-e-k-e	89	1245
4237	4252	528938	TAGGTGTTCCCATACG	e-e-e-d(10)-k-k-k	72	336
4237	4252	530094	TAGGTGTTCCCATACG	e-k-k-d(10)-k-k-e	88	336
4237	4252	530141	TAGGTGTTCCCATACG	e-e-k-d(10)-k-k-e	80	336
4237	4252	530191	TAGGTGTTCCCATACG	e-d-k-d(10)-k-k-e	74	336
4237	4252	530241	TAGGTGTTCCCATACG	e-d-d-k-d(9)-k-k-e	53	336
4237	4252	530291	TAGGTGTTCCCATACG	e-e-e-e-d(9)-k-k-e	68	336
4238	4253	528939	CTAGGTGTTCCCATAC	e-e-e-d(10)-k-k-k	39	1247
4239	4254	528940	GCTAGGTGTTCCCATA	e-e-e-d(10)-k-k-k	62	1248
4240	4255	528941	TGCTAGGTGTTCCCAT	e-e-e-d(10)-k-k-k	49	1249
4242	4257	528942	CGTGCTAGGTGTTCCC	e-e-e-d(10)-k-k-k	77	1250
4304	4319	528943	CAAGGTGGTTTTGAGT	e-e-e-d(10)-k-k-k	25	1251
4305	4320	528944	GCAAGGTGGTTTTGAG	e-e-e-d(10)-k-k-k	28	344
4320	4335	528945	CTCTGATCAGCTGAGG	e-e-e-d(10)-k-k-k	74	1252
4321	4336	528946	ACTCTGATCAGCTGAG	e-e-e-d(10)-k-k-k	56	1253
4362	4377	528947	GAGACCAGCTAATTTG	e-e-e-d(10)-k-k-k	36	1254
4395	4410	528948	CATCTTAGAGAAGGTC	e-e-e-d(10)-k-k-k	59	1255
4435	4450	528949	TCAACTGTCTCCAGGC	e-e-e-d(10)-k-k-k	67	1256
4435	4450	530397	TCAACTGTCTCCAGGC	k-d(10)-k-e-k-e-e	60	1256
4436	4451	528950	ATCAACTGTCTCCAGG	e-e-e-d(10)-k-k-k	57	1257
4436	4452	530027	CATCAACTGTCTCCAGG	e-e-k-d(10)-k-e-k-e	56	1258
4436	4451	530347	ATCAACTGTCTCCAGG	e-k-d(10)-k-e-k-e	49	1257
4437	4452	530095	CATCAACTGTCTCCAG	e-k-k-d(10)-k-k-e	40	354
4437	4452	530142	CATCAACTGTCTCCAG	e-e-k-d(10)-k-k-e	43	354
4437	4452	530192	CATCAACTGTCTCCAG	e-d-k-d(10)-k-k-e	42	354
4437	4452	530242	CATCAACTGTCTCCAG	e-d-d-k-d(9)-k-k-e	0	354
4437	4452	530292	CATCAACTGTCTCCAG	e-e-e-e-d(9)-k-k-e	36	354
4437	4452	530398	CATCAACTGTCTCCAG	k-d(10)-k-e-k-e-e	28	354
4438	4454	530028	CACATCAACTGTCTCCA	e-e-k-d(10)-k-e-k-e	57	1259
4438	4453	530348	ACATCAACTGTCTCCA	e-k-d(10)-k-e-k-e	58	1260
4439	4454	530096	CACATCAACTGTCTCC	e-k-k-d(10)-k-k-e	72	356
4439	4454	530143	CACATCAACTGTCTCC	e-e-k-d(10)-k-k-e	74	356
4439	4454	530193	CACATCAACTGTCTCC	e-d-k-d(10)-k-k-e	62	356
4439	4454	530243	CACATCAACTGTCTCC	e-d-d-k-d(9)-k-k-e	34	356
4439	4454	530293	CACATCAACTGTCTCC	e-e-e-e-d(9)-k-k-e	59	356

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4441	4456	528951	GACACATCAACTGTCT	e-e-e-d(10)-k-k-k	16	1261
4475	4490	528952	GAAGAGTGTGCTGGA	e-e-e-d(10)-k-k-k	57	1262
4477	4492	528953	CTGAAGAGTGTGCTG	e-e-e-d(10)-k-k-k	46	1263
4479	4494	528954	TACTGAAGAGTGTGTC	e-e-e-d(10)-k-k-k	42	1264
4485	4500	530510	ATTATGTACTGAAGAG	k-d(10)-k-e-k-e-e	53	1265
4486	4501	530504	TATTATGTACTGAAGA	e-k-d(10)-k-e-k-e	25	1266
4486	4501	530511	TATTATGTACTGAAGA	k-d(10)-k-e-k-e-e	31	1266
4487	4502	530432	TTATTATGTACTGAAG	k-d(10)-k-e-k-e-e	15	1267
4487	4502	530463	TTATTATGTACTGAAG	e-k-k-d(10)-k-k-e	20	1267
4487	4502	530472	TTATTATGTACTGAAG	e-e-k-d(10)-k-k-e	17	1267
4487	4502	530480	TTATTATGTACTGAAG	e-d-k-d(10)-k-k-e	4	1267
4487	4502	530488	TTATTATGTACTGAAG	e-d-d-k-d(9)-k-k-e	13	1267
4487	4502	530496	TTATTATGTACTGAAG	e-e-e-e-d(9)-k-k-e	0	1267
4487	4502	530505	TTATTATGTACTGAAG	e-k-d(10)-k-e-k-e	37	1267
4488	4504	530063	GCTTATTATGTACTGAA	e-e-k-d(10)-k-e-k-e	74	1268
4488	4503	530382	CTTATTATGTACTGAA	e-k-d(10)-k-e-k-e	17	1269
4488	4503	530465	CTTATTATGTACTGAA	e-k-k-d(10)-k-k-e	63	1269
4488	4503	530473	CTTATTATGTACTGAA	e-e-k-d(10)-k-k-e	45	1269
4488	4503	530481	CTTATTATGTACTGAA	e-d-k-d(10)-k-k-e	14	1269
4488	4503	530489	CTTATTATGTACTGAA	e-d-d-k-d(9)-k-k-e	13	1269
4488	4503	530497	CTTATTATGTACTGAA	e-e-e-e-d(9)-k-k-e	7	1269
4488	4503	530512	CTTATTATGTACTGAA	k-d(10)-k-e-k-e-e	21	1269
4489	4504	519638	GCTTATTATGTACTGA	e-k-k-d(10)-k-k-e	86	362
4489	4504	530177	GCTTATTATGTACTGA	e-e-k-d(10)-k-k-e	71	362
4489	4504	530227	GCTTATTATGTACTGA	e-d-k-d(10)-k-k-e	51	362
4489	4504	530277	GCTTATTATGTACTGA	e-d-d-k-d(9)-k-k-e	70	362
4489	4504	530327	GCTTATTATGTACTGA	e-e-e-e-d(9)-k-k-e	61	362
4490	4505	530466	AGCTTATTATGTACTG	e-k-k-d(10)-k-k-e	82	1270
4490	4505	530474	AGCTTATTATGTACTG	e-e-k-d(10)-k-k-e	62	1270
4490	4505	530482	AGCTTATTATGTACTG	e-d-k-d(10)-k-k-e	53	1270
4490	4505	530490	AGCTTATTATGTACTG	e-d-d-k-d(9)-k-k-e	42	1270
4490	4505	530498	AGCTTATTATGTACTG	e-e-e-e-d(9)-k-k-e	45	1270
4490	4505	530506	AGCTTATTATGTACTG	e-k-d(10)-k-e-k-e	70	1270
4491	4506	530467	AAGCTTATTATGTACT	e-k-k-d(10)-k-k-e	50	1271
4491	4506	530475	AAGCTTATTATGTACT	e-e-k-d(10)-k-k-e	26	1271
4491	4506	530483	AAGCTTATTATGTACT	e-d-k-d(10)-k-k-e	19	1271
4491	4506	530491	AAGCTTATTATGTACT	e-d-d-k-d(9)-k-k-e	13	1271
4491	4506	530499	AAGCTTATTATGTACT	e-e-e-e-d(9)-k-k-e	15	1271
4492	4507	528955	TAAGCTTATTATGTAC	e-e-e-d(10)-k-k-k	0	1272
4499	4514	528956	TATCAGTTAAGCTTAT	e-e-e-d(10)-k-k-k	0	1273
4502	4517	528957	GTTTATCAGTTAAGCT	e-e-e-d(10)-k-k-k	31	1274
4539	4554	530433	CAATGGTAAGCCCAAG	k-d(10)-k-e-k-e-e	62	1275

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4540	4555	528958	CCAATGGTAAGCCCAA	e-e-e-d(10)-k-k-k	66	1276
4540	4556	530056	CCCAATGGTAAGCCCAA	e-e-k-d(10)-k-e-k-e	73	1277
4540	4555	530383	CCAATGGTAAGCCCAA	e-k-d(10)-k-e-k-e	64	1276
4541	4556	518345	CCCAATGGTAAGCCCA	e-e-e-d(10)-k-k-k	80	366
4541	4556	519636	CCCAATGGTAAGCCCA	e-k-k-d(10)-k-k-e	90	366
4541	4556	530178	CCCAATGGTAAGCCCA	e-e-k-d(10)-k-k-e	86	366
4541	4556	530228	CCCAATGGTAAGCCCA	e-d-k-d(10)-k-k-e	77	366
4541	4556	530278	CCCAATGGTAAGCCCA	e-d-d-k-d(9)-k-k-e	86	366
4541	4556	530328	CCCAATGGTAAGCCCA	e-e-e-e-d(9)-k-k-e	80	366
4542	4557	528959	ACCCAATGGTAAGCCC	e-e-e-d(10)-k-k-k	73	1277
4544	4559	528960	AAACCCAATGGTAAGC	e-e-e-d(10)-k-k-k	43	1278
4545	4560	528961	TAAACCCAATGGTAAG	e-e-e-d(10)-k-k-k	18	1279
4546	4561	528962	TTAAACCCAATGGTAA	e-e-e-d(10)-k-k-k	13	1280
4547	4562	528963	TTTAAACCCAATGGTA	e-e-e-d(10)-k-k-k	2	1281
4554	4569	528964	CCTATGATTTAAACCC	e-e-e-d(10)-k-k-k	17	1282
4558	4573	528965	GGTCCCTATGATTTAA	e-e-e-d(10)-k-k-k	31	1283
4559	4574	528966	AGGTCCCTATGATTTA	e-e-e-d(10)-k-k-k	22	1284
4615	4630	528967	CCTAAGGCCATGAACT	e-e-e-d(10)-k-k-k	19	374
4616	4631	528968	ACCTAAGGCCATGAAC	e-e-e-d(10)-k-k-k	25	1285
4617	4632	528969	TACCTAAGGCCATGAA	e-e-e-d(10)-k-k-k	41	1286
4618	4633	528970	CTACCTAAGGCCATGA	e-e-e-d(10)-k-k-k	55	1287
4619	4634	528971	GCTACCTAAGGCCATG	e-e-e-d(10)-k-k-k	66	1288
4620	4635	528972	TGCTACCTAAGGCCAT	e-e-e-d(10)-k-k-k	56	1289
4621	4636	528973	ATGCTACCTAAGGCCA	e-e-e-d(10)-k-k-k	71	1290
4622	4637	528974	CATGCTACCTAAGGCC	e-e-e-d(10)-k-k-k	58	1291
4623	4638	528975	ACATGCTACCTAAGGC	e-e-e-d(10)-k-k-k	34	1292
4636	4651	528976	GTTAAGACCAGATACA	e-e-e-d(10)-k-k-k	45	1293
4637	4652	528977	AGTTAAGACCAGATAC	e-e-e-d(10)-k-k-k	40	1294
4638	4653	528978	GAGTTAAGACCAGATA	e-e-e-d(10)-k-k-k	40	1295
4639	4654	528979	AGAGTTAAGACCAGAT	e-e-e-d(10)-k-k-k	62	1296
4644	4659	530399	CAATCAGAGTTAAGAC	k-d(10)-k-e-k-e-e	36	1297
4645	4661	530029	TACAATCAGAGTTAAGA	e-e-k-d(10)-k-e-k-e	29	1298
4645	4660	530349	ACAATCAGAGTTAAGA	e-k-d(10)-k-e-k-e	33	1299
4646	4661	528980	TACAATCAGAGTTAAG	e-e-e-d(10)-k-k-k	0	378
4646	4661	530097	TACAATCAGAGTTAAG	e-k-k-d(10)-k-k-e	41	378
4646	4661	530144	TACAATCAGAGTTAAG	e-e-k-d(10)-k-k-e	16	378
4646	4661	530194	TACAATCAGAGTTAAG	e-d-k-d(10)-k-k-e	28	378
4646	4661	530244	TACAATCAGAGTTAAG	e-d-d-k-d(9)-k-k-e	0	378
4646	4661	530294	TACAATCAGAGTTAAG	e-e-e-e-d(9)-k-k-e	7	378
4648	4663	528981	GCTACAATCAGAGTTA	e-e-e-d(10)-k-k-k	52	1300
4649	4664	528982	TGCTACAATCAGAGTT	e-e-e-d(10)-k-k-k	47	1301
4650	4665	528983	TTGCTACAATCAGAGT	e-e-e-d(10)-k-k-k	44	1302

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4662	4677	530400	CTCTCAGAACTTTTGC	k-d(10)-k-e-k-e-e	65	1303
4663	4679	530030	TCCTCTCAGAACTTTTG	e-e-k-d(10)-k-e-k-e	47	1304
4663	4678	530350	CCTCTCAGAACTTTTG	e-k-d(10)-k-e-k-e	54	1305
4664	4679	530098	TCCTCTCAGAACTTTT	e-k-k-d(10)-k-k-e	42	380
4664	4679	530145	TCCTCTCAGAACTTTT	e-e-k-d(10)-k-k-e	38	380
4664	4679	530195	TCCTCTCAGAACTTTT	e-d-k-d(10)-k-k-e	43	380
4664	4679	530245	TCCTCTCAGAACTTTT	e-d-d-k-d(9)-k-k-e	28	380
4664	4679	530295	TCCTCTCAGAACTTTT	e-e-e-e-d(9)-k-k-e	39	380
4770	4785	528984	CCCACGGGATTCCCTC	e-e-e-d(10)-k-k-k	39	1306
4771	4786	528985	ACCCACGGGATTCCCT	e-e-e-d(10)-k-k-k	36	1307
4772	4787	528986	AACCCACGGGATTCCC	e-e-e-d(10)-k-k-k	47	1308
4773	4788	528987	CAACCCACGGGATTCC	e-e-e-d(10)-k-k-k	39	1309
4774	4789	528988	GCAACCCACGGGATTC	e-e-e-d(10)-k-k-k	48	1310
4775	4790	528989	AGCAACCCACGGGATT	e-e-e-d(10)-k-k-k	40	1311
4777	4792	528990	TAAGCAACCCACGGGA	e-e-e-d(10)-k-k-k	27	1312
4778	4793	528991	GTAAGCAACCCACGGG	e-e-e-d(10)-k-k-k	47	1313
4779	4794	528992	GGTAAGCAACCCACGG	e-e-e-d(10)-k-k-k	42	1314
4780	4795	528993	AGGTAAGCAACCCACG	e-e-e-d(10)-k-k-k	54	1315
4780	4795	530434	AGGTAAGCAACCCACG	k-d(10)-k-e-k-e-e	51	1315
4781	4796	528994	TAGGTAAGCAACCCAC	e-e-e-d(10)-k-k-k	53	1316
4781	4797	530064	GTAGGTAAGCAACCCAC	e-e-k-d(10)-k-e-k-e	53	1317
4781	4796	530384	TAGGTAAGCAACCCAC	e-k-d(10)-k-e-k-e	48	1316
4782	4797	528995	GTAGGTAAGCAACCCA	e-e-e-d(10)-k-k-k	64	388
4782	4797	530129	GTAGGTAAGCAACCCA	e-k-k-d(10)-k-k-e	79	388
4782	4797	530179	GTAGGTAAGCAACCCA	e-e-k-d(10)-k-k-e	74	388
4782	4797	530229	GTAGGTAAGCAACCCA	e-d-k-d(10)-k-k-e	64	388
4782	4797	530279	GTAGGTAAGCAACCCA	e-d-d-k-d(9)-k-k-e	55	388
4782	4797	530329	GTAGGTAAGCAACCCA	e-e-e-e-d(9)-k-k-e	61	388
4784	4799	528996	AGGTAGGTAAGCAACC	e-e-e-d(10)-k-k-k	21	1318
4788	4803	528997	TTATAGGTAGGTAAGC	e-e-e-d(10)-k-k-k	10	1319
4792	4807	528998	CACCTTATAGGTAGGT	e-e-e-d(10)-k-k-k	22	1320
4794	4809	528999	ACCACCTTATAGGTAG	e-e-e-d(10)-k-k-k	15	1321
4797	4812	529000	TAAACCACCTTATAGG	e-e-e-d(10)-k-k-k	0	1322
4798	4813	529001	ATAAACCACCTTATAG	e-e-e-d(10)-k-k-k	7	1323
4810	4825	529002	GGACAGCAGCTTATAA	e-e-e-d(10)-k-k-k	12	1324
4811	4826	529003	AGGACAGCAGCTTATA	e-e-e-d(10)-k-k-k	40	1325
4811	4826	530401	AGGACAGCAGCTTATA	k-d(10)-k-e-k-e-e	41	1325
4812	4827	529004	CAGGACAGCAGCTTAT	e-e-e-d(10)-k-k-k	38	1326
4812	4828	530031	CCAGGACAGCAGCTTAT	e-e-k-d(10)-k-e-k-e	58	1327
4812	4827	530351	CAGGACAGCAGCTTAT	e-k-d(10)-k-e-k-e	58	1326
4812	4827	530402	CAGGACAGCAGCTTAT	k-d(10)-k-e-k-e-e	60	1326
4813	4829	530032	GCCAGGACAGCAGCTTA	e-e-k-d(10)-k-e-k-e	74	1328

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4813	4828	530099	CCAGGACAGCAGCTTA	e-k-k-d(10)-k-k-e	73	1329
4813	4828	530146	CCAGGACAGCAGCTTA	e-e-k-d(10)-k-k-e	70	1329
4813	4828	530196	CCAGGACAGCAGCTTA	e-d-k-d(10)-k-k-e	67	1329
4813	4828	530246	CCAGGACAGCAGCTTA	e-d-d-k-d(9)-k-k-e	39	1329
4813	4828	530296	CCAGGACAGCAGCTTA	e-e-e-e-d(9)-k-k-e	67	1329
4813	4828	530352	CCAGGACAGCAGCTTA	e-k-d(10)-k-e-k-e	67	1329
4814	4829	530100	GCCAGGACAGCAGCTT	e-k-k-d(10)-k-k-e	77	1330
4814	4829	530147	GCCAGGACAGCAGCTT	e-e-k-d(10)-k-k-e	84	1330
4814	4829	530197	GCCAGGACAGCAGCTT	e-d-k-d(10)-k-k-e	71	1330
4814	4829	530247	GCCAGGACAGCAGCTT	e-d-d-k-d(9)-k-k-e	53	1330
4814	4829	530297	GCCAGGACAGCAGCTT	e-e-e-e-d(9)-k-k-e	75	1330
4814	4829	530403	GCCAGGACAGCAGCTT	k-d(10)-k-e-k-e-e	77	1330
4815	4831	530033	TGGCCAGGACAGCAGCT	e-e-k-d(10)-k-e-k-e	65	1331
4815	4830	530353	GGCCAGGACAGCAGCT	e-k-d(10)-k-e-k-e	83	1332
4816	4831	530101	TGGCCAGGACAGCAGC	e-k-k-d(10)-k-k-e	59	1333
4816	4831	530148	TGGCCAGGACAGCAGC	e-e-k-d(10)-k-k-e	79	1333
4816	4831	530198	TGGCCAGGACAGCAGC	e-d-k-d(10)-k-k-e	54	1333
4816	4831	530248	TGGCCAGGACAGCAGC	e-d-d-k-d(9)-k-k-e	32	1333
4816	4831	530298	TGGCCAGGACAGCAGC	e-e-e-e-d(9)-k-k-e	73	1333
4827	4842	530404	TTTGAATGCAGTGGCC	k-d(10)-k-e-k-e-e	67	1334
4828	4844	530034	AATTTGAATGCAGTGGC	e-e-k-d(10)-k-e-k-e	69	1335
4828	4843	530354	ATTTGAATGCAGTGGC	e-k-d(10)-k-e-k-e	85	1336
4828	4843	530405	ATTTGAATGCAGTGGC	k-d(10)-k-e-k-e-e	55	1336
4829	4845	530035	GAATTTGAATGCAGTGG	e-e-k-d(10)-k-e-k-e	69	1337
4829	4844	530102	AATTTGAATGCAGTGG	e-k-k-d(10)-k-k-e	71	1338
4829	4844	530149	AATTTGAATGCAGTGG	e-e-k-d(10)-k-k-e	70	1338
4829	4844	530199	AATTTGAATGCAGTGG	e-d-k-d(10)-k-k-e	58	1338
4829	4844	530249	AATTTGAATGCAGTGG	e-d-d-k-d(9)-k-k-e	47	1338
4829	4844	530299	AATTTGAATGCAGTGG	e-e-e-e-d(9)-k-k-e	47	1338
4829	4844	530355	AATTTGAATGCAGTGG	e-k-d(10)-k-e-k-e	72	1338
4830	4845	530103	GAATTTGAATGCAGTG	e-k-k-d(10)-k-k-e	77	390
4830	4845	530150	GAATTTGAATGCAGTG	e-e-k-d(10)-k-k-e	73	390
4830	4845	530200	GAATTTGAATGCAGTG	e-d-k-d(10)-k-k-e	63	390
4830	4845	530250	GAATTTGAATGCAGTG	e-d-d-k-d(9)-k-k-e	59	390
4830	4845	530300	GAATTTGAATGCAGTG	e-e-e-e-d(9)-k-k-e	65	390
4842	4857	530435	AAGTACACATTGGAAT	k-d(10)-k-e-k-e-e	62	1339
4843	4859	530057	TGAAGTACACATTGGAA	e-e-k-d(10)-k-e-k-e	69	1340
4843	4858	530385	GAAGTACACATTGGAA	e-k-d(10)-k-e-k-e	70	1341
4844	4859	529005	TGAAGTACACATTGGA	e-e-e-d(10)-k-k-k	64	392
4844	4859	530130	TGAAGTACACATTGGA	e-k-k-d(10)-k-k-e	85	392
4844	4859	530180	TGAAGTACACATTGGA	e-e-k-d(10)-k-k-e	82	392
4844	4859	530230	TGAAGTACACATTGGA	e-d-k-d(10)-k-k-e	65	392

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4844	4859	530280	TGAAGTACACATTGGA	e-d-d-k-d(9)-k-k-e	75	392
4844	4859	530330	TGAAGTACACATTGGA	e-e-e-d(9)-k-k-e	52	392
4852	4867	529006	TTACACTATGAAGTAC	e-e-e-d(10)-k-k-k	16	1342
4929	4944	529007	AGTTAAAGTAGATACA	e-e-e-d(10)-k-k-k	0	1343
4934	4949	529008	CTGGAAGTTAAAGTAG	e-e-e-d(10)-k-k-k	30	397
4943	4958	529009	CGTTTATTTCTGGAAG	e-e-e-d(10)-k-k-k	52	1344
4957	4972	529010	CGGTCCTATATAACG	e-e-e-d(10)-k-k-k	21	1345
4958	4973	529011	ACGGTCCTATATAAC	e-e-e-d(10)-k-k-k	10	1346

Table 14

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 2

Human Start Site	Human Stop Site	ISIS No	Sequence	Chemistry	% inhibition	SEQ ID NO
1359	1374	529012	GTCATCCCGAAGAGTC	e-e-e-d(10)-k-k-k	34	1347
1386	1401	529013	CCCGAGTCCCTCCGA	e-e-e-d(10)-k-k-k	18	1348
1390	1405	529014	GCGCCCCGAGTCCCTT	e-e-e-d(10)-k-k-k	53	1349
1412	1427	529015	CGAAGAACGAACTTC	e-e-e-d(10)-k-k-k	8	1350
1418	1433	529016	TTTCTCCGAAGAACGA	e-e-e-d(10)-k-k-k	31	1351
1461	1476	529017	CGAGTGCGCCCTCGCC	e-e-e-d(10)-k-k-k	52	1352
1548	1563	529018	GTGACAGTCGCTCCGG	e-e-e-d(10)-k-k-k	30	1353
1549	1564	529019	CGTGACAGTCGCTCCG	e-e-e-d(10)-k-k-k	31	1354
1590	1605	529020	GCGCTTCCGACCCCC	e-e-e-d(10)-k-k-k	45	1355
1790	1805	529021	GTACCGGTCTGTCAAT	e-e-e-d(10)-k-k-k	23	1356
1794	1809	529022	AAGAGTACCGGTCTGT	e-e-e-d(10)-k-k-k	69	1357
1796	1811	529023	GAAAGAGTACCGGTCT	e-e-e-d(10)-k-k-k	72	1358
1906	1921	529024	CTGGCTTGACGGGTTG	e-e-e-d(10)-k-k-k	64	1359
1907	1922	529025	GCTGGCTTGACGGGTT	e-e-e-d(10)-k-k-k	73	1360
1966	1981	529026	CCGACTTTACCAGGTA	e-e-e-d(10)-k-k-k	78	1361
1968	1983	529027	GGCCGACTTTACCAGG	e-e-e-d(10)-k-k-k	92	1362
1972	1987	529028	TTCTGGCCGACTTTAC	e-e-e-d(10)-k-k-k	13	1363
2031	2046	529029	CGTCCTATGCAATTAA	e-e-e-d(10)-k-k-k	24	1364
2039	2054	529030	GTTTCATCCGTCCTAT	e-e-e-d(10)-k-k-k	41	1365
2198	2213	529031	GACGGTTTGAATCTTG	e-e-e-d(10)-k-k-k	40	1366
2201	2216	529032	GGCGACGGTTTGAATC	e-e-e-d(10)-k-k-k	37	1367
2204	2219	529033	TTGGGCGACGGTTTGA	e-e-e-d(10)-k-k-k	31	1368
2207	2222	529034	AACTTGGGCGACGGTT	e-e-e-d(10)-k-k-k	54	1369
2253	2268	529035	CGACCTGATATGGCAC	e-e-e-d(10)-k-k-k	56	1370
2255	2270	529036	AACGACCTGATATGGC	e-e-e-d(10)-k-k-k	52	1371
2257	2272	529037	ACAACGACCTGATATG	e-e-e-d(10)-k-k-k	24	1372
2338	2353	530406	ATACAGTAAGACCAGC	k-d(10)-k-e-k-e-e	65	1373
2339	2355	530036	ACATACAGTAAGACCAG	e-e-k-d(10)-k-e-k-e	58	1374
2339	2354	530356	CATACAGTAAGACCAG	e-k-d(10)-k-e-k-e	65	1375

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2340	2355	530104	ACATACAGTAAGACCA	e-k-k-d(10)-k-k-e	67	1376
2340	2355	530151	ACATACAGTAAGACCA	e-e-k-d(10)-k-k-e	64	1376
2340	2355	530201	ACATACAGTAAGACCA	e-d-k-d(10)-k-k-e	42	1376
2340	2355	530251	ACATACAGTAAGACCA	e-d-d-k-d(9)-k-k-e	58	1376
2340	2355	530301	ACATACAGTAAGACCA	e-e-e-e-d(9)-k-k-e	56	1376
2383	2398	530407	AAAATTTACAACCCAT	k-d(10)-k-e-k-e-e	9	1377
2384	2400	530037	CAAAAATTTACAACCCA	e-e-k-d(10)-k-e-k-e	42	1378
2384	2399	530357	AAAAATTTACAACCCA	e-k-d(10)-k-e-k-e	34	1379
2385	2400	530105	CAAAAATTTACAACCC	e-k-k-d(10)-k-k-e	40	1380
2385	2400	530152	CAAAAATTTACAACCC	e-e-k-d(10)-k-k-e	33	1380
2385	2400	530202	CAAAAATTTACAACCC	e-d-k-d(10)-k-k-e	10	1380
2385	2400	530252	CAAAAATTTACAACCC	e-d-d-k-d(9)-k-k-e	29	1380
2385	2400	530302	CAAAAATTTACAACCC	e-e-e-e-d(9)-k-k-e	14	1380
2408	2423	530408	AATGCTTTATCAGCAC	k-d(10)-k-e-k-e-e	36	1381
2409	2425	530038	CCAATGCTTTATCAGCA	e-e-k-d(10)-k-e-k-e	71	1382
2409	2424	530358	CAATGCTTTATCAGCA	e-k-d(10)-k-e-k-e	46	1383
2410	2425	530106	CCAATGCTTTATCAGC	e-k-k-d(10)-k-k-e	70	1384
2410	2425	530153	CCAATGCTTTATCAGC	e-e-k-d(10)-k-k-e	50	1384
2410	2425	530203	CCAATGCTTTATCAGC	e-d-k-d(10)-k-k-e	43	1384
2410	2425	530253	CCAATGCTTTATCAGC	e-d-d-k-d(9)-k-k-e	33	1384
2410	2425	530303	CCAATGCTTTATCAGC	e-e-e-e-d(9)-k-k-e	40	1384
2669	2684	530409	ACTAAAATCAAGGCTC	k-d(10)-k-e-k-e-e	42	1385
2670	2686	530039	AGACTAAAATCAAGGCT	e-e-k-d(10)-k-e-k-e	73	1386
2670	2685	530359	GACTAAAATCAAGGCT	e-k-d(10)-k-e-k-e	82	1387
2671	2686	530107	AGACTAAAATCAAGGC	e-k-k-d(10)-k-k-e	77	1388
2671	2686	530154	AGACTAAAATCAAGGC	e-e-k-d(10)-k-k-e	57	1388
2671	2686	530204	AGACTAAAATCAAGGC	e-d-k-d(10)-k-k-e	28	1388
2671	2686	530254	AGACTAAAATCAAGGC	e-d-d-k-d(9)-k-k-e	3	1388
2671	2686	530304	AGACTAAAATCAAGGC	e-e-e-e-d(9)-k-k-e	22	1388
2703	2718	530429	AATGGTTCTTTGTGAT	k-d(10)-k-e-k-e-e	60	1389
2704	2720	530065	CTAATGGTTCTTTGTGA	e-e-k-d(10)-k-e-k-e	70	1390
2704	2719	530379	TAATGGTTCTTTGTGA	e-k-d(10)-k-e-k-e	54	1391
2705	2720	530127	CTAATGGTTCTTTGTG	e-k-k-d(10)-k-k-e	80	411
2705	2720	530174	CTAATGGTTCTTTGTG	e-e-k-d(10)-k-k-e	69	411
2705	2720	530224	CTAATGGTTCTTTGTG	e-d-k-d(10)-k-k-e	32	411
2705	2720	530274	CTAATGGTTCTTTGTG	e-d-d-k-d(9)-k-k-e	38	411
2705	2720	530324	CTAATGGTTCTTTGTG	e-e-e-e-d(9)-k-k-e	32	411
5000	5015	530410	CTGAAATTCCTTGGTC	k-d(10)-k-e-k-e-e	53	1392
5001	5017	530040	AACTGAAATTCCTTGGT	e-e-k-d(10)-k-e-k-e	67	1393
5001	5016	530360	ACTGAAATTCCTTGGT	e-k-d(10)-k-e-k-e	70	1394
5002	5017	530108	AACTGAAATTCCTTGG	e-k-k-d(10)-k-k-e	70	1395
5002	5017	530155	AACTGAAATTCCTTGG	e-e-k-d(10)-k-k-e	53	1395

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5002	5017	530205	AACTGAAATTCCTTGG	e-d-k-d(10)-k-k-e	44	1395
5002	5017	530255	AACTGAAATTCCTTGG	e-d-d-k-d(9)-k-k-e	33	1395
5002	5017	530305	AACTGAAATTCCTTGG	e-e-e-e-d(9)-k-k-e	22	1395
5699	5714	530411	ACTCTTTCAGTGGTTT	k-d(10)-k-e-k-e-e	91	1396
5700	5716	530041	GTACTCTTTCAGTGGTT	e-e-k-d(10)-k-e-k-e	89	1397
5700	5715	530361	TACTCTTTCAGTGGTT	e-k-d(10)-k-e-k-e	88	1398
5701	5716	530109	GTACTCTTTCAGTGGT	e-k-k-d(10)-k-k-e	89	1399
5701	5716	530156	GTACTCTTTCAGTGGT	e-e-k-d(10)-k-k-e	91	1399
5701	5716	530206	GTACTCTTTCAGTGGT	e-d-k-d(10)-k-k-e	89	1399
5701	5716	530256	GTACTCTTTCAGTGGT	e-d-d-k-d(9)-k-k-e	33	1399
5701	5716	530306	GTACTCTTTCAGTGGT	e-e-e-e-d(9)-k-k-e	83	1399
5883	5898	529038	CTACACTTTACGCTTA	e-e-e-d(10)-k-k-k	9	1400
6474	6489	530436	AATTCATTCTTCCATA	k-d(10)-k-e-k-e-e	49	1401
6475	6491	530066	GAAATTCATTCTTCCAT	e-e-k-d(10)-k-e-k-e	82	1402
6475	6490	530386	AAATTCATTCTTCCAT	e-k-d(10)-k-e-k-e	53	1403
6476	6491	530131	GAAATTCATTCTTCCA	e-k-k-d(10)-k-k-e	97	413
6476	6491	530181	GAAATTCATTCTTCCA	e-e-k-d(10)-k-k-e	82	413
6476	6491	530231	GAAATTCATTCTTCCA	e-d-k-d(10)-k-k-e	75	413
6476	6491	530281	GAAATTCATTCTTCCA	e-d-d-k-d(9)-k-k-e	69	413
6476	6491	530331	GAAATTCATTCTTCCA	e-e-e-e-d(9)-k-k-e	53	413
6846	6861	529039	TTAAAGAGTTGCGGTA	e-e-e-d(10)-k-k-k	31	1404
6847	6862	529040	ATTAAAGAGTTGCGGT	e-e-e-d(10)-k-k-k	34	1405
8078	8093	530412	AGATTTACCTTCCTTA	k-d(10)-k-e-k-e-e	50	1406
8079	8095	530042	GCAGATTTACCTTCCTT	e-e-k-d(10)-k-e-k-e	78	1407
8079	8094	530362	CAGATTTACCTTCCTT	e-k-d(10)-k-e-k-e	76	1408
8080	8095	530110	GCAGATTTACCTTCCT	e-k-k-d(10)-k-k-e	84	1409
8080	8095	530157	GCAGATTTACCTTCCT	e-e-k-d(10)-k-k-e	69	1409
8080	8095	530207	GCAGATTTACCTTCCT	e-d-k-d(10)-k-k-e	55	1409
8080	8095	530257	GCAGATTTACCTTCCT	e-d-d-k-d(9)-k-k-e	39	1409
8080	8095	530307	GCAGATTTACCTTCCT	e-e-e-e-d(9)-k-k-e	77	1409
9123	9138	530413	GCCCCCTATGTATAAGC	k-d(10)-k-e-k-e-e	73	1410
9124	9140	530043	CTGCCCCCTATGTATAAG	e-e-k-d(10)-k-e-k-e	42	1411
9124	9139	530363	TGCCCCCTATGTATAAG	e-k-d(10)-k-e-k-e	25	1412
9125	9140	530111	CTGCCCCCTATGTATAA	e-k-k-d(10)-k-k-e	35	1413
9125	9140	530158	CTGCCCCCTATGTATAA	e-e-k-d(10)-k-k-e	36	1413
9125	9140	530208	CTGCCCCCTATGTATAA	e-d-k-d(10)-k-k-e	14	1413
9125	9140	530258	CTGCCCCCTATGTATAA	e-d-d-k-d(9)-k-k-e	5	1413
9125	9140	530308	CTGCCCCCTATGTATAA	e-e-e-e-d(9)-k-k-e	25	1413
9862	9877	530414	TTCTTCCTGAGACACA	k-d(10)-k-e-k-e-e	61	1414
9863	9879	530044	GCTTCTTCCTGAGACAC	e-e-k-d(10)-k-e-k-e	78	1415
9863	9878	530364	CTTCTTCCTGAGACAC	e-k-d(10)-k-e-k-e	59	1416
9864	9879	530112	GCTTCTTCCTGAGACA	e-k-k-d(10)-k-k-e	84	1417

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9864	9879	530159	GCTTCTTCCTGAGACA	e-e-k-d(10)-k-k-e	69	1417
9864	9879	530209	GCTTCTTCCTGAGACA	e-d-k-d(10)-k-k-e	54	1417
9864	9879	530259	GCTTCTTCCTGAGACA	e-d-d-k-d(9)-k-k-e	57	1417
9864	9879	530309	GCTTCTTCCTGAGACA	e-e-e-e-d(9)-k-k-e	46	1417
9864	9879	530415	GCTTCTTCCTGAGACA	k-d(10)-k-e-k-e-e	51	1417
9865	9881	530045	TGGCTTCTTCCTGAGAC	e-e-k-d(10)-k-e-k-e	73	1418
9865	9880	530365	GGCTTCTTCCTGAGAC	e-k-d(10)-k-e-k-e	78	1419
9866	9881	530113	TGGCTTCTTCCTGAGA	e-k-k-d(10)-k-k-e	60	1420
9866	9881	530160	TGGCTTCTTCCTGAGA	e-e-k-d(10)-k-k-e	54	1420
9866	9881	530210	TGGCTTCTTCCTGAGA	e-d-k-d(10)-k-k-e	28	1420
9866	9881	530260	TGGCTTCTTCCTGAGA	e-d-d-k-d(9)-k-k-e	0	1420
9866	9881	530310	TGGCTTCTTCCTGAGA	e-e-e-e-d(9)-k-k-e	26	1420
9873	9888	530416	CTCCTGTTGGCTTCTT	k-d(10)-k-e-k-e-e	57	1421
9874	9890	530046	TCCTCCTGTTGGCTTCT	e-e-k-d(10)-k-e-k-e	76	1422
9874	9889	530366	CCTCCTGTTGGCTTCT	e-k-d(10)-k-e-k-e	75	1423
9874	9889	530417	CCTCCTGTTGGCTTCT	k-d(10)-k-e-k-e-e	66	1423
9875	9891	530047	TTCTCCTGTTGGCTTC	e-e-k-d(10)-k-e-k-e	75	1424
9875	9890	530114	TCCTCCTGTTGGCTTC	e-k-k-d(10)-k-k-e	80	1425
9875	9890	530161	TCCTCCTGTTGGCTTC	e-e-k-d(10)-k-k-e	81	1425
9875	9890	530211	TCCTCCTGTTGGCTTC	e-d-k-d(10)-k-k-e	73	1425
9875	9890	530261	TCCTCCTGTTGGCTTC	e-d-d-k-d(9)-k-k-e	78	1425
9875	9890	530311	TCCTCCTGTTGGCTTC	e-e-e-e-d(9)-k-k-e	82	1425
9875	9890	530367	TCCTCCTGTTGGCTTC	e-k-d(10)-k-e-k-e	80	1425
9876	9891	530115	TTCCTCCTGTTGGCTT	e-k-k-d(10)-k-k-e	74	1426
9876	9891	530162	TTCCTCCTGTTGGCTT	e-e-k-d(10)-k-k-e	68	1426
9876	9891	530212	TTCCTCCTGTTGGCTT	e-d-k-d(10)-k-k-e	58	1426
9876	9891	530262	TTCCTCCTGTTGGCTT	e-d-d-k-d(9)-k-k-e	23	1426
9876	9891	530312	TTCCTCCTGTTGGCTT	e-e-e-e-d(9)-k-k-e	52	1426
9876	9891	530418	TTCCTCCTGTTGGCTT	k-d(10)-k-e-k-e-e	59	1426
9877	9893	530048	GGTTCCTCCTGTTGGCT	e-e-k-d(10)-k-e-k-e	82	1427
9877	9892	530368	GTTCTCCTGTTGGCT	e-k-d(10)-k-e-k-e	85	1428
9878	9893	530116	GGTTCCTCCTGTTGGC	e-k-k-d(10)-k-k-e	90	1429
9878	9893	530163	GGTTCCTCCTGTTGGC	e-e-k-d(10)-k-k-e	79	1429
9878	9893	530213	GGTTCCTCCTGTTGGC	e-d-k-d(10)-k-k-e	72	1429
9878	9893	530263	GGTTCCTCCTGTTGGC	e-d-d-k-d(9)-k-k-e	73	1429
9878	9893	530313	GGTTCCTCCTGTTGGC	e-e-e-e-d(9)-k-k-e	61	1429
9964	9979	529041	GTAATGTGCAGCAATC	e-e-e-d(10)-k-k-k	53	1430
9991	10006	530711	ATGTGAGGGCACATTT	e-e-e-d(10)-k-k-k	25	1431
10286	10301	529042	CCAAGCCGTTTATTTT	e-e-e-d(10)-k-k-k	44	1432
10291	10306	529043	GGAAGCCAAGCCGTTT	e-e-e-d(10)-k-k-k	39	1433
11261	11276	530413	GCCCCTATGTATAAGC	k-d(10)-k-e-k-e-e	73	1410
11262	11278	530043	CTGCCCTATGTATAAG	e-e-k-d(10)-k-e-k-e	42	1411

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11262	11277	530363	TGCCCCCTATGTATAAG	e-k-d(10)-k-e-k-e	25	1412
11263	11278	530111	CTGCCCCCTATGTATAA	e-k-k-d(10)-k-k-e	35	1413
11263	11278	530158	CTGCCCCCTATGTATAA	e-e-k-d(10)-k-k-e	36	1413
11263	11278	530208	CTGCCCCCTATGTATAA	e-d-k-d(10)-k-k-e	14	1413
11263	11278	530258	CTGCCCCCTATGTATAA	e-d-d-k-d(9)-k-k-e	5	1413
11263	11278	530308	CTGCCCCCTATGTATAA	e-e-e-e-d(9)-k-k-e	25	1413
12345	12360	530414	TTCTTCCTGAGACACA	k-d(10)-k-e-k-e-e	61	1414
12346	12362	530044	GCTTCTTCCTGAGACAC	e-e-k-d(10)-k-e-k-e	78	1415
12346	12361	530364	CTTCTTCCTGAGACAC	e-k-d(10)-k-e-k-e	59	1416
12347	12362	530112	GCTTCTTCCTGAGACA	e-k-k-d(10)-k-k-e	84	1417
12347	12362	530159	GCTTCTTCCTGAGACA	e-e-k-d(10)-k-k-e	69	1417
12347	12362	530209	GCTTCTTCCTGAGACA	e-d-k-d(10)-k-k-e	54	1417
12347	12362	530259	GCTTCTTCCTGAGACA	e-d-d-k-d(9)-k-k-e	57	1417
12347	12362	530309	GCTTCTTCCTGAGACA	e-e-e-e-d(9)-k-k-e	46	1417
12347	12362	530415	GCTTCTTCCTGAGACA	k-d(10)-k-e-k-e-e	51	1417
12348	12364	530045	TGGCTTCTTCCTGAGAC	e-e-k-d(10)-k-e-k-e	73	1418
12348	12363	530365	GGCTTCTTCCTGAGAC	e-k-d(10)-k-e-k-e	78	1419
12349	12364	530113	TGGCTTCTTCCTGAGA	e-k-k-d(10)-k-k-e	60	1420
12349	12364	530160	TGGCTTCTTCCTGAGA	e-e-k-d(10)-k-k-e	54	1420
12349	12364	530210	TGGCTTCTTCCTGAGA	e-d-k-d(10)-k-k-e	28	1420
12349	12364	530260	TGGCTTCTTCCTGAGA	e-d-d-k-d(9)-k-k-e	0	1420
12349	12364	530310	TGGCTTCTTCCTGAGA	e-e-e-e-d(9)-k-k-e	26	1420
12356	12371	530416	CTCCTGTTGGCTTCTT	k-d(10)-k-e-k-e-e	57	1421
12357	12373	530046	TCCTCCTGTTGGCTTCT	e-e-k-d(10)-k-e-k-e	76	1422
12357	12372	530366	CCTCCTGTTGGCTTCT	e-k-d(10)-k-e-k-e	75	1423
12357	12372	530417	CCTCCTGTTGGCTTCT	k-d(10)-k-e-k-e-e	66	1423
12358	12374	530047	TTCTCCTGTTGGCTTC	e-e-k-d(10)-k-e-k-e	75	1424
12358	12373	530114	TCCTCCTGTTGGCTTC	e-k-k-d(10)-k-k-e	80	1425
12358	12373	530161	TCCTCCTGTTGGCTTC	e-e-k-d(10)-k-k-e	81	1425
12358	12373	530211	TCCTCCTGTTGGCTTC	e-d-k-d(10)-k-k-e	73	1425
12358	12373	530261	TCCTCCTGTTGGCTTC	e-d-d-k-d(9)-k-k-e	78	1425
12358	12373	530311	TCCTCCTGTTGGCTTC	e-e-e-e-d(9)-k-k-e	82	1425
12358	12373	530367	TCCTCCTGTTGGCTTC	e-k-d(10)-k-e-k-e	80	1425
12359	12374	530115	TTCTCCTGTTGGCTT	e-k-k-d(10)-k-k-e	74	1426
12359	12374	530162	TTCTCCTGTTGGCTT	e-e-k-d(10)-k-k-e	68	1426
12359	12374	530212	TTCTCCTGTTGGCTT	e-d-k-d(10)-k-k-e	58	1426
12359	12374	530262	TTCTCCTGTTGGCTT	e-d-d-k-d(9)-k-k-e	23	1426
12359	12374	530312	TTCTCCTGTTGGCTT	e-e-e-e-d(9)-k-k-e	52	1426
12359	12374	530418	TTCTCCTGTTGGCTT	k-d(10)-k-e-k-e-e	59	1426
12360	12376	530048	GGTTCCTCCTGTTGGCT	e-e-k-d(10)-k-e-k-e	82	1427
12360	12375	530368	GTTCTCCTGTTGGCT	e-k-d(10)-k-e-k-e	85	1428
12361	12376	530116	GGTTCCTCCTGTTGGC	e-k-k-d(10)-k-k-e	90	1429

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12361	12376	530163	GGTTCCTCCTGTTGGC	e-e-k-d(10)-k-k-e	79	1429
12361	12376	530213	GGTTCCTCCTGTTGGC	e-d-k-d(10)-k-k-e	72	1429
12361	12376	530263	GGTTCCTCCTGTTGGC	e-d-d-k-d(9)-k-k-e	73	1429
12361	12376	530313	GGTTCCTCCTGTTGGC	e-e-e-e-d(9)-k-k-e	61	1429
12586	12601	530710	TACAATTCCTGCCTGT	e-e-e-d(10)-k-k-k	18	1434
15467	15482	530437	AGCTTTTCTATGAAAA	k-d(10)-k-e-k-e-e	5	1435
15468	15484	530067	CAAGCTTTTCTATGAAA	e-e-k-d(10)-k-e-k-e	53	1436
15468	15483	530387	AAGCTTTTCTATGAAA	e-k-d(10)-k-e-k-e	24	1437
15469	15484	530132	CAAGCTTTTCTATGAA	e-k-k-d(10)-k-k-e	74	423
15469	15484	530182	CAAGCTTTTCTATGAA	e-e-k-d(10)-k-k-e	48	423
15469	15484	530232	CAAGCTTTTCTATGAA	e-d-k-d(10)-k-k-e	21	423
15469	15484	530282	CAAGCTTTTCTATGAA	e-d-d-k-d(9)-k-k-e	19	423
15469	15484	530332	CAAGCTTTTCTATGAA	e-e-e-e-d(9)-k-k-e	20	423
16863	16878	530419	TAATTGTGTACTGGCA	k-d(10)-k-e-k-e-e	75	1438
16864	16880	530049	TATAATTGTGTACTGGC	e-e-k-d(10)-k-e-k-e	88	1439
16864	16879	530369	ATAATTGTGTACTGGC	e-k-d(10)-k-e-k-e	92	1440
16865	16880	530117	TATAATTGTGTACTGG	e-k-k-d(10)-k-k-e	73	1441
16865	16880	530164	TATAATTGTGTACTGG	e-e-k-d(10)-k-k-e	65	1441
16865	16880	530214	TATAATTGTGTACTGG	e-d-k-d(10)-k-k-e	37	1441
16865	16880	530264	TATAATTGTGTACTGG	e-d-d-k-d(9)-k-k-e	48	1441
16865	16880	530314	TATAATTGTGTACTGG	e-e-e-e-d(9)-k-k-e	42	1441
17385	17400	530709	TGGAGTAACAGGAAGT	e-e-e-d(10)-k-k-k	25	1442
21456	21471	530720	AAAGTTTCCCAATAGA	e-e-e-d(10)-k-k-k	17	1443
22061	22076	529044	AGTCCTACCACGGCCC	e-e-e-d(10)-k-k-k	27	1444
24514	24529	529045	TGACGATGCTTGGATA	e-e-e-d(10)-k-k-k	37	1445
24515	24530	529046	CTGACGATGCTTGGAT	e-e-e-d(10)-k-k-k	8	1446
24579	24594	529047	TCACTTTCCTATACG	e-e-e-d(10)-k-k-k	18	1447
25105	25120	530717	GTAGGTTGAGCAAGCA	e-e-e-d(10)-k-k-k	77	1448
26061	26076	530420	ACTTTAGCCCCTTCCA	k-d(10)-k-e-k-e-e	44	1449
26062	26078	530050	CAACTTTAGCCCCTTCC	e-e-k-d(10)-k-e-k-e	64	1450
26062	26077	530370	AACTTTAGCCCCTTCC	e-k-d(10)-k-e-k-e	55	1451
26063	26078	530118	CAACTTTAGCCCCTTC	e-k-k-d(10)-k-k-e	58	1452
26063	26078	530165	CAACTTTAGCCCCTTC	e-e-k-d(10)-k-k-e	38	1452
26063	26078	530215	CAACTTTAGCCCCTTC	e-d-k-d(10)-k-k-e	29	1452
26063	26078	530265	CAACTTTAGCCCCTTC	e-d-d-k-d(9)-k-k-e	3	1452
26063	26078	530315	CAACTTTAGCCCCTTC	e-e-e-e-d(9)-k-k-e	30	1452
26767	26782	529048	AATTCATCGAGCTAAT	e-e-e-d(10)-k-k-k	0	1453
37758	37773	529049	TGCCCCAATTAGGCCA	e-e-e-d(10)-k-k-k	32	1454
37759	37774	529050	TTGCCCCAATTAGGCC	e-e-e-d(10)-k-k-k	21	1455
41484	41499	530714	CCCTGTGGCTCCTTCC	e-e-e-d(10)-k-k-k	27	1456
41760	41775	529051	TACTGTCTCGAGACA	e-e-e-d(10)-k-k-k	2	1457
42754	42769	530719	AGGAAAAGGAAGAATG	e-e-e-d(10)-k-k-k	2	1458

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42766	42781	529052	CGCATATGCCCTAGGA	e-e-e-d(10)-k-k-k	7	1459
42768	42783	529053	GCCGCATATGCCCTAG	e-e-e-d(10)-k-k-k	41	1460
42769	42784	529054	GGCCGCATATGCCCTA	e-e-e-d(10)-k-k-k	51	1461
43072	43087	529055	CGGGTAAGTATACAGA	e-e-e-d(10)-k-k-k	18	1462
43074	43089	529056	CACGGGTAAGTATACA	e-e-e-d(10)-k-k-k	4	1463
43075	43090	529057	TCACGGGTAAGTATAC	e-e-e-d(10)-k-k-k	5	1464
43077	43092	529058	GCTCACGGGTAAGTAT	e-e-e-d(10)-k-k-k	15	1465
45633	45648	529059	GTATACAATGGCCTTT	e-e-e-d(10)-k-k-k	59	1466
46633	46648	529060	CGACCCAATCAGATGC	e-e-e-d(10)-k-k-k	34	1467
47430	47445	530708	GGATAAAATACAAAGG	e-e-e-d(10)-k-k-k	14	1468
47617	47632	529061	GTTCCGAAAAAACCTC	e-e-e-d(10)-k-k-k	59	1469
47619	47634	529062	GGGTTCCGAAAAAACCC	e-e-e-d(10)-k-k-k	16	1470
47752	47767	530712	TGCAAACCTTTTCTCT	e-e-e-d(10)-k-k-k	21	1471
48092	48107	529063	ACCCGCTATCCACTCA	e-e-e-d(10)-k-k-k	20	1472
48402	48417	530421	CACCTTCCATTCTAGT	k-d(10)-k-e-k-e-e	20	1473
48403	48419	530051	CACACTTCCATTCTAG	e-e-k-d(10)-k-e-k-e	48	1474
48403	48418	530371	ACACTTCCATTCTAG	e-k-d(10)-k-e-k-e	36	1475
48404	48419	530119	CACACTTCCATTCTA	e-k-k-d(10)-k-k-e	47	1476
48404	48419	530166	CACACTTCCATTCTA	e-e-k-d(10)-k-k-e	53	1476
48404	48419	530216	CACACTTCCATTCTA	e-d-k-d(10)-k-k-e	34	1476
48404	48419	530266	CACACTTCCATTCTA	e-d-d-k-d(9)-k-k-e	31	1476
48404	48419	530316	CACACTTCCATTCTA	e-e-e-e-d(9)-k-k-e	34	1476
48429	48444	529064	AGCCCCTATGGTTACC	e-e-e-d(10)-k-k-k	32	1477
48567	48582	529065	GTCTAGAGGCCTATCC	e-e-e-d(10)-k-k-k	14	1478
48568	48583	529066	GGTCTAGAGGCCTATC	e-e-e-d(10)-k-k-k	17	1479
49762	49777	530718	AGATGTTGGATGTCTA	e-e-e-d(10)-k-k-k	46	1480
50692	50707	530423	AGATTCTCTACCACTT	k-d(10)-k-e-k-e-e	70	1054
50693	50709	530053	GGAGATTCTCTACCACT	e-e-k-d(10)-k-e-k-e	84	1055
50693	50708	530373	GAGATTCTCTACCACT	e-k-d(10)-k-e-k-e	85	1056
50694	50709	530121	GGAGATTCTCTACCAC	e-k-k-d(10)-k-k-e	77	53
50694	50709	530168	GGAGATTCTCTACCAC	e-e-k-d(10)-k-k-e	75	53
50694	50709	530218	GGAGATTCTCTACCAC	e-d-k-d(10)-k-k-e	61	53
50694	50709	530268	GGAGATTCTCTACCAC	e-d-d-k-d(9)-k-k-e	76	53
50694	50709	530318	GGAGATTCTCTACCAC	e-e-e-e-d(9)-k-k-e	73	53
50838	50853	529067	CCGCCTTAAGATCTAA	e-e-e-d(10)-k-k-k	5	1481
51714	51729	529068	CCCTTACTCTCCGCAT	e-e-e-d(10)-k-k-k	15	1482
51734	51749	529069	GGGAAGTGGTCCGACC	e-e-e-d(10)-k-k-k	22	1483
51757	51772	529070	CCGCAAGTGAGCGAGA	e-e-e-d(10)-k-k-k	6	1484
51760	51775	529071	ATCCCGCAAGTGAGCG	e-e-e-d(10)-k-k-k	11	1485
51763	51778	529072	GAAATCCCGCAAGTGA	e-e-e-d(10)-k-k-k	0	1486
51905	51920	528400	CCGCCAGCTCACTCAC	e-e-e-d(10)-k-k-k	57	66
51906	51921	528401	CCCGCCAGCTCACTCA	e-e-e-d(10)-k-k-k	57	1059

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51907	51922	528402	CCCCGCCAGCTCACTC	e-e-e-d(10)-k-k-k	42	1060
51910	51925	528403	AAGCCCCGCCAGCTCA	e-e-e-d(10)-k-k-k	72	1060
51911	51926	528404	AAAGCCCCGCCAGCTC	e-e-e-d(10)-k-k-k	52	1062
51912	51927	528405	AAAAGCCCCGCCAGCT	e-e-e-d(10)-k-k-k	27	1063
51913	51928	528406	CAAAAGCCCCGCCAGC	e-e-e-d(10)-k-k-k	29	1064
51914	51929	528407	ACAAAAGCCCCGCCAG	e-e-e-d(10)-k-k-k	9	1065
51916	51931	528408	TGACAAAAGCCCCGCC	e-e-e-d(10)-k-k-k	10	1066
51917	51932	528409	CTGACAAAAGCCCCGC	e-e-e-d(10)-k-k-k	31	1067
51918	51933	528410	GCTGACAAAAGCCCCG	e-e-e-d(10)-k-k-k	39	1068
51919	51934	528411	CGCTGACAAAAGCCCC	e-e-e-d(10)-k-k-k	49	1069
51920	51935	528412	TCGCTGACAAAAGCCC	e-e-e-d(10)-k-k-k	39	1070
51921	51936	528413	ATCGCTGACAAAAGCC	e-e-e-d(10)-k-k-k	20	1071
51922	51937	528414	CATCGCTGACAAAAGC	e-e-e-d(10)-k-k-k	10	1072
51924	51939	528415	TCCATCGCTGACAAAA	e-e-e-d(10)-k-k-k	11	1073
51925	51940	528416	CTCCATCGCTGACAAA	e-e-e-d(10)-k-k-k	15	1074
51926	51941	528417	ACTCCATCGCTGACAA	e-e-e-d(10)-k-k-k	22	1075
51927	51942	528418	TACTCCATCGCTGACA	e-e-e-d(10)-k-k-k	19	1076
51928	51943	528419	GTACTCCATCGCTGAC	e-e-e-d(10)-k-k-k	37	1077
51929	51944	528420	CGTACTCCATCGCTGA	e-e-e-d(10)-k-k-k	35	1078
51943	51958	528421	GAGAGTTTTCTGCACG	e-e-e-d(10)-k-k-k	36	1079
51945	51960	528422	GTGAGAGTTTTCTGCA	e-e-e-d(10)-k-k-k	22	1080
51964	51979	528423	GTCAGCCAGCTCCTCG	e-e-e-d(10)-k-k-k	49	1081
51975	51990	528424	CGCCTCTTCCAGTCAG	e-e-e-d(10)-k-k-k	42	1082
51977	51992	528425	GCCGCCTCTTCCAGTC	e-e-e-d(10)-k-k-k	44	1083
51978	51993	528426	TGCCGCCTCTTCCAGT	e-e-e-d(10)-k-k-k	15	1084
51983	51998	528427	TCTGTTGCCGCCTCTT	e-e-e-d(10)-k-k-k	9	1085
51984	51999	528428	ATCTGTTGCCGCCTCT	e-e-e-d(10)-k-k-k	30	1086
51985	52000	528429	AATCTGTTGCCGCCTC	e-e-e-d(10)-k-k-k	23	1087
51986	52001	528430	CAATCTGTTGCCGCCT	e-e-e-d(10)-k-k-k	12	1088
51987	52002	528431	GCAATCTGTTGCCGCC	e-e-e-d(10)-k-k-k	48	1089
51988	52003	528432	GGCAATCTGTTGCCGC	e-e-e-d(10)-k-k-k	18	1090
51989	52004	528433	AGGCAATCTGTTGCCG	e-e-e-d(10)-k-k-k	0	1091
51990	52005	528434	CAGGCAATCTGTTGCC	e-e-e-d(10)-k-k-k	8	1092
51991	52006	528435	GCAGGCAATCTGTTGC	e-e-e-d(10)-k-k-k	13	1093
51995	52010	528436	CAATGCAGGCAATCTG	e-e-e-d(10)-k-k-k	9	1094
51996	52011	528437	CCAATGCAGGCAATCT	e-e-e-d(10)-k-k-k	26	1095
51997	52012	528438	TCCAATGCAGGCAATC	e-e-e-d(10)-k-k-k	10	1096
51998	52013	528439	CTCCAATGCAGGCAAT	e-e-e-d(10)-k-k-k	2	1097
51999	52014	528440	CCTCCAATGCAGGCAA	e-e-e-d(10)-k-k-k	28	1098
52016	52031	528441	GGCAGATGTTGGGCGG	e-e-e-d(10)-k-k-k	8	1099
52017	52032	528442	AGGCAGATGTTGGGCG	e-e-e-d(10)-k-k-k	0	1100
52018	52033	528443	TAGGCAGATGTTGGGC	e-e-e-d(10)-k-k-k	1	1101

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52019	52034	528444	CTAGGCAGATGTTGGG	e-e-e-d(10)-k-k-k	0	1102
52020	52035	528445	TCTAGGCAGATGTTGG	e-e-e-d(10)-k-k-k	7	1103
52021	52036	528446	ATCTAGGCAGATGTTG	e-e-e-d(10)-k-k-k	3	1104
52023	52038	528447	CGATCTAGGCAGATGT	e-e-e-d(10)-k-k-k	9	72
52024	52039	528448	CCGATCTAGGCAGATG	e-e-e-d(10)-k-k-k	13	1105
52026	52041	528449	AGCCGATCTAGGCAGA	e-e-e-d(10)-k-k-k	4	1106
52027	52042	528450	TAGCCGATCTAGGCAG	e-e-e-d(10)-k-k-k	11	1107
52028	52043	528451	CTAGCCGATCTAGGCA	e-e-e-d(10)-k-k-k	5	1108
52029	52044	528452	TCTAGCCGATCTAGGC	e-e-e-d(10)-k-k-k	5	1109
52030	52045	528453	TTCTAGCCGATCTAGG	e-e-e-d(10)-k-k-k	24	1110
52031	52046	528454	TTTCTAGCCGATCTAG	e-e-e-d(10)-k-k-k	29	1111
52032	52047	528455	TTTTCTAGCCGATCTA	e-e-e-d(10)-k-k-k	28	1112
52033	52048	528456	GTTTTCTAGCCGATCT	e-e-e-d(10)-k-k-k	42	1113
52035	52050	528457	CAGTTTTCTAGCCGAT	e-e-e-d(10)-k-k-k	50	1114
52036	52051	528458	CCAGTTTTCTAGCCGA	e-e-e-d(10)-k-k-k	70	1115
52083	52098	529073	TCAATCTAGCTTTCGA	e-e-e-d(10)-k-k-k	33	1487
52084	52099	529074	TTCAATCTAGCTTTCG	e-e-e-d(10)-k-k-k	36	1488
52119	52134	529075	GTACCAATTCTGTGGG	e-e-e-d(10)-k-k-k	33	1489
55441	55456	528462	GATTCTGCTAATGACG	e-e-e-d(10)-k-k-k	42	1119
55442	55457	528463	AGATTCTGCTAATGAC	e-e-e-d(10)-k-k-k	38	1120
55446	55461	528464	GTTGAGATTCTGCTAA	e-e-e-d(10)-k-k-k	30	1121
55447	55462	528465	AGTTGAGATTCTGCTA	e-e-e-d(10)-k-k-k	48	1122
55454	55469	528466	GGTCTGAAGTTGAGAT	e-e-e-d(10)-k-k-k	27	1123
55456	55471	528467	CGGGTCTGAAGTTGAG	e-e-e-d(10)-k-k-k	44	1124
55457	55472	528468	ACGGGTCTGAAGTTGA	e-e-e-d(10)-k-k-k	41	1125
55458	55473	528469	GACGGGTCTGAAGTTG	e-e-e-d(10)-k-k-k	45	1126
55459	55474	528470	TGACGGGTCTGAAGTT	e-e-e-d(10)-k-k-k	34	1127
55460	55475	528471	TTGACGGGTCTGAAGT	e-e-e-d(10)-k-k-k	19	1128
55461	55476	528472	GTTGACGGGTCTGAAG	e-e-e-d(10)-k-k-k	21	1129
55462	55477	528473	TGTTGACGGGTCTGAA	e-e-e-d(10)-k-k-k	37	1130
55463	55478	528474	TTGTTGACGGGTCTGA	e-e-e-d(10)-k-k-k	55	1131
55464	55479	528475	TTTGTGACGGGTCTG	e-e-e-d(10)-k-k-k	63	1132
55465	55480	528476	ATTTGTTGACGGGTCT	e-e-e-d(10)-k-k-k	65	1133
56208	56223	529076	GTAACACCTCACCTA	e-e-e-d(10)-k-k-k	14	1490
58396	58411	530715	TCTGCCACCCAGGTTT	e-e-e-d(10)-k-k-k	31	1491
59836	59851	529077	TAAATTTCCGGGATCT	e-e-e-d(10)-k-k-k	13	1492
64187	64202	529078	CCGGTCCCTTGTA AAA	e-e-e-d(10)-k-k-k	12	1493
64289	64304	529079	GCCAACTCTAGGCGAG	e-e-e-d(10)-k-k-k	16	1494
64551	64566	529080	CGCAAGAGATCCCGGG	e-e-e-d(10)-k-k-k	0	1495
64552	64567	529081	TCGCAAGAGATCCCGG	e-e-e-d(10)-k-k-k	16	1496
64959	64974	529082	TGATCACCTCGACTGA	e-e-e-d(10)-k-k-k	20	1497
66136	66151	530425	GCCCTTGCCAGCCATG	k-d(10)-k-e-k-e-e	73	1134

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66137	66153	530054	AAGCCCTTGCCAGCCAT	e-e-k-d(10)-k-e-k-e	75	1135
66137	66152	530375	AGCCCTTGCCAGCCAT	e-k-d(10)-k-e-k-e	77	1136
66138	66153	530123	AAGCCCTTGCCAGCCA	e-k-k-d(10)-k-k-e	86	144
66138	66153	530170	AAGCCCTTGCCAGCCA	e-e-k-d(10)-k-k-e	87	144
66138	66153	530220	AAGCCCTTGCCAGCCA	e-d-k-d(10)-k-k-e	74	144
66138	66153	530270	AAGCCCTTGCCAGCCA	e-d-d-k-d(9)-k-k-e	87	144
66138	66153	530320	AAGCCCTTGCCAGCCA	e-e-e-e-d(9)-k-k-e	83	144
66183	66198	530426	TTTTTCACAAGGTCAA	k-d(10)-k-e-k-e-e	55	1137
66184	66200	530059	ACTTTTTCACAAGGTCA	e-e-k-d(10)-k-e-k-e	73	1138
66184	66199	530376	CTTTTTCACAAGGTCA	e-k-d(10)-k-e-k-e	77	1139
66185	66200	530124	ACTTTTTCACAAGGTC	e-k-k-d(10)-k-k-e	79	153
66185	66200	530171	ACTTTTTCACAAGGTC	e-e-k-d(10)-k-k-e	69	153
66185	66200	530221	ACTTTTTCACAAGGTC	e-d-k-d(10)-k-k-e	64	153
66185	66200	530271	ACTTTTTCACAAGGTC	e-d-d-k-d(9)-k-k-e	73	153
66185	66200	530321	ACTTTTTCACAAGGTC	e-e-e-e-d(9)-k-k-e	56	153
66875	66890	529083	GCCACCCTAGTGTTGA	e-e-e-d(10)-k-k-k	27	1498
67066	67081	530427	ATGATCTTATAGCCCA	k-d(10)-k-e-k-e-e	43	931
67067	67083	530060	CCATGATCTTATAGCCC	e-e-k-d(10)-k-e-k-e	77	1140
67067	67082	530377	CATGATCTTATAGCCC	e-k-d(10)-k-e-k-e	66	932
67068	67083	530125	CCATGATCTTATAGCC	e-k-k-d(10)-k-k-e	65	175
67068	67083	530172	CCATGATCTTATAGCC	e-e-k-d(10)-k-k-e	59	175
67068	67083	530222	CCATGATCTTATAGCC	e-d-k-d(10)-k-k-e	48	175
67068	67083	530272	CCATGATCTTATAGCC	e-d-d-k-d(9)-k-k-e	63	175
67068	67083	530322	CCATGATCTTATAGCC	e-e-e-e-d(9)-k-k-e	45	175
67270	67285	530716	TTTGCCATCTATCCT	e-e-e-d(10)-k-k-k	11	1499
67346	67361	529084	CGGTCACCCCAACAAA	e-e-e-d(10)-k-k-k	33	1500
69470	69485	529085	AAGGGCGATGGTAATG	e-e-e-d(10)-k-k-k	4	1501
71614	71629	530422	GTACAATTGCTTCAAC	k-d(10)-k-e-k-e-e	46	1502
71615	71631	530052	CAGTACAATTGCTTCAA	e-e-k-d(10)-k-e-k-e	51	1503
71615	71630	530372	AGTACAATTGCTTCAA	e-k-d(10)-k-e-k-e	51	1504
71616	71631	530120	CAGTACAATTGCTTCA	e-k-k-d(10)-k-k-e	78	1505
71616	71631	530167	CAGTACAATTGCTTCA	e-e-k-d(10)-k-k-e	69	1505
71616	71631	530217	CAGTACAATTGCTTCA	e-d-k-d(10)-k-k-e	47	1505
71616	71631	530267	CAGTACAATTGCTTCA	e-d-d-k-d(9)-k-k-e	64	1505
71616	71631	530317	CAGTACAATTGCTTCA	e-e-e-e-d(9)-k-k-e	60	1505
72138	72153	530713	CTCATGCCAAGATTGT	e-e-e-d(10)-k-k-k	26	1506
72299	72314	529086	AAGCCACTTACGGTGT	e-e-e-d(10)-k-k-k	0	1507
72874	72889	529087	CGTCTATTTCAGTGT	e-e-e-d(10)-k-k-k	22	1508
73648	73663	529088	ACTAGTTCAGTTGTCC	e-e-e-d(10)-k-k-k	0	1509
73866	73881	530428	TAGCAGAAGTAGGAGA	k-d(10)-k-e-k-e-e	49	1141
73867	73883	530061	GATAGCAGAAGTAGGAG	e-e-k-d(10)-k-e-k-e	49	1142
73867	73882	530378	ATAGCAGAAGTAGGAG	e-k-d(10)-k-e-k-e	48	1143

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73868	73883	530126	GATAGCAGAAGTAGGA	e-k-k-d(10)-k-k-e	70	223
73868	73883	530173	GATAGCAGAAGTAGGA	e-e-k-d(10)-k-k-e	62	223
73868	73883	530223	GATAGCAGAAGTAGGA	e-d-k-d(10)-k-k-e	44	223
73868	73883	530273	GATAGCAGAAGTAGGA	e-d-d-k-d(9)-k-k-e	63	223
73868	73883	530323	GATAGCAGAAGTAGGA	e-e-e-e-d(9)-k-k-e	37	223
74199	74214	530513	TTGGATGTCAGCAAGG	k-d(10)-k-e-k-e-e	88	1047
74200	74215	530507	TTTGGATGTCAGCAAG	e-k-d(10)-k-e-k-e	86	1144
74200	74215	530514	TTTGGATGTCAGCAAG	k-d(10)-k-e-k-e-e	80	1144
74201	74216	530430	ATTTGGATGTCAGCAA	k-d(10)-k-e-k-e-e	87	1145
74201	74216	530468	ATTTGGATGTCAGCAA	e-k-k-d(10)-k-k-e	81	1145
74201	74216	530476	ATTTGGATGTCAGCAA	e-e-k-d(10)-k-k-e	82	1145
74201	74216	530484	ATTTGGATGTCAGCAA	e-d-k-d(10)-k-k-e	74	1145
74201	74216	530492	ATTTGGATGTCAGCAA	e-d-d-k-d(9)-k-k-e	83	1145
74201	74216	530500	ATTTGGATGTCAGCAA	e-e-e-e-d(9)-k-k-e	56	1145
74201	74216	530508	ATTTGGATGTCAGCAA	e-k-d(10)-k-e-k-e	83	1145
74202	74218	530062	CTATTTGGATGTCAGCA	e-e-k-d(10)-k-e-k-e	94	1146
74202	74217	530380	TATTTGGATGTCAGCA	e-k-d(10)-k-e-k-e	94	1147
74202	74217	530469	TATTTGGATGTCAGCA	e-k-k-d(10)-k-k-e	91	1147
74202	74217	530477	TATTTGGATGTCAGCA	e-e-k-d(10)-k-k-e	87	1147
74202	74217	530485	TATTTGGATGTCAGCA	e-d-k-d(10)-k-k-e	87	1147
74202	74217	530493	TATTTGGATGTCAGCA	e-d-d-k-d(9)-k-k-e	81	1147
74202	74217	530501	TATTTGGATGTCAGCA	e-e-e-e-d(9)-k-k-e	74	1147
74202	74217	530515	TATTTGGATGTCAGCA	k-d(10)-k-e-k-e-e	87	1147
74203	74218	481464	CTATTTGGATGTCAGC	k-k-k-d(10)-k-k-k	93	245
74203	74218	518349	CTATTTGGATGTCAGC	e-e-e-d(10)-k-k-k	58	245
74203	74218	519637	CTATTTGGATGTCAGC	e-k-k-d(10)-k-k-e	96	245
74203	74218	530175	CTATTTGGATGTCAGC	e-e-k-d(10)-k-k-e	93	245
74203	74218	530225	CTATTTGGATGTCAGC	e-d-k-d(10)-k-k-e	85	245
74203	74218	530275	CTATTTGGATGTCAGC	e-d-d-k-d(9)-k-k-e	91	245
74203	74218	530325	CTATTTGGATGTCAGC	e-e-e-e-d(9)-k-k-e	91	245
74204	74219	530470	TCTATTTGGATGTCAG	e-k-k-d(10)-k-k-e	91	1148
74204	74219	530478	TCTATTTGGATGTCAG	e-e-k-d(10)-k-k-e	87	1148
74204	74219	530486	TCTATTTGGATGTCAG	e-d-k-d(10)-k-k-e	84	1148
74204	74219	530494	TCTATTTGGATGTCAG	e-d-d-k-d(9)-k-k-e	60	1148
74204	74219	530502	TCTATTTGGATGTCAG	e-e-e-e-d(9)-k-k-e	64	1148
74204	74219	530509	TCTATTTGGATGTCAG	e-k-d(10)-k-e-k-e	80	1148
74205	74220	530471	TTCTATTTGGATGTCA	e-k-k-d(10)-k-k-e	83	1149
74205	74220	530479	TTCTATTTGGATGTCA	e-e-k-d(10)-k-k-e	74	1149
74205	74220	530487	TTCTATTTGGATGTCA	e-d-k-d(10)-k-k-e	71	1149
74205	74220	530495	TTCTATTTGGATGTCA	e-d-d-k-d(9)-k-k-e	68	1149
74205	74220	530503	TTCTATTTGGATGTCA	e-e-e-e-d(9)-k-k-e	53	1149
74646	74661	530431	CACCAAGGAGGCTGTT	k-d(10)-k-e-k-e-e	44	1150

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74647	74663	530055	AGCACCAAGGAGGCTGT	e-e-k-d(10)-k-e-k-e	45	1151
74647	74662	530381	GCACCAAGGAGGCTGT	e-k-d(10)-k-e-k-e	74	1152
74648	74663	530128	AGCACCAAGGAGGCTG	e-k-k-d(10)-k-k-e	52	257
74648	74663	530176	AGCACCAAGGAGGCTG	e-e-k-d(10)-k-k-e	66	257
74648	74663	530226	AGCACCAAGGAGGCTG	e-d-k-d(10)-k-k-e	51	257
74648	74663	530276	AGCACCAAGGAGGCTG	e-d-d-k-d(9)-k-k-e	70	257
74648	74663	530326	AGCACCAAGGAGGCTG	e-e-e-e-d(9)-k-k-e	52	257
74714	74729	528860	GGTTTGACCTGAAGCC	e-e-e-d(10)-k-k-k	58	1153
74715	74730	528861	GGTTTGACCTGAAGC	e-e-e-d(10)-k-k-k	42	1154
74716	74731	528862	AGGGTTTGACCTGAAG	e-e-e-d(10)-k-k-k	57	1155
74717	74732	528863	AAGGGTTTGACCTGAA	e-e-e-d(10)-k-k-k	43	1156
74718	74733	528864	TAAGGGTTTGACCTGA	e-e-e-d(10)-k-k-k	50	1157
74719	74734	528865	TTAAGGGTTTGACCTG	e-e-e-d(10)-k-k-k	32	1158
74734	74749	528866	GCAGCTTCAGATGTCT	e-e-e-d(10)-k-k-k	60	1159
74735	74750	528867	TGCAGCTTCAGATGTC	e-e-e-d(10)-k-k-k	47	1160
74770	74785	530388	CTTAAACCTTCCTATT	k-d(10)-k-e-k-e-e	14	1161
74771	74786	530338	CCTTAAACCTTCCTAT	e-k-d(10)-k-e-k-e	47	1162
74772	74787	530086	TCCTTAAACCTTCCTA	e-k-k-d(10)-k-k-e	58	273
74772	74787	530133	TCCTTAAACCTTCCTA	e-e-k-d(10)-k-k-e	53	273
74772	74787	530183	TCCTTAAACCTTCCTA	e-d-k-d(10)-k-k-e	52	273
74772	74787	530233	TCCTTAAACCTTCCTA	e-d-d-k-d(9)-k-k-e	29	273
74772	74787	530283	TCCTTAAACCTTCCTA	e-e-e-e-d(9)-k-k-e	32	273
74777	74792	528868	GATTCTCCTTAAACCT	e-e-e-d(10)-k-k-k	45	1163
74778	74793	530389	AGATTCTCCTTAAACC	k-d(10)-k-e-k-e-e	44	1164
74779	74794	530339	TAGATTCTCCTTAAAC	e-k-d(10)-k-e-k-e	41	1165
74780	74795	530087	TTAGATTCTCCTTAAA	e-k-k-d(10)-k-k-e	43	1166
74780	74795	530134	TTAGATTCTCCTTAAA	e-e-k-d(10)-k-k-e	28	1166
74780	74795	530184	TTAGATTCTCCTTAAA	e-d-k-d(10)-k-k-e	13	1166
74780	74795	530234	TTAGATTCTCCTTAAA	e-d-d-k-d(9)-k-k-e	15	1166
74780	74795	530284	TTAGATTCTCCTTAAA	e-e-e-e-d(9)-k-k-e	14	1166
74782	74797	530390	GCTTAGATTCTCCTTA	k-d(10)-k-e-k-e-e	83	1167
74783	74798	530340	TGCTTAGATTCTCCTT	e-k-d(10)-k-e-k-e	89	1168
74784	74799	528869	ATGCTTAGATTCTCCT	e-e-e-d(10)-k-k-k	83	1169
74784	74799	530088	ATGCTTAGATTCTCCT	e-k-k-d(10)-k-k-e	90	1169
74784	74799	530135	ATGCTTAGATTCTCCT	e-e-k-d(10)-k-k-e	91	1169
74784	74799	530185	ATGCTTAGATTCTCCT	e-d-k-d(10)-k-k-e	85	1169
74784	74799	530235	ATGCTTAGATTCTCCT	e-d-d-k-d(9)-k-k-e	28	1169
74784	74799	530285	ATGCTTAGATTCTCCT	e-e-e-e-d(9)-k-k-e	86	1169
74784	74799	530391	ATGCTTAGATTCTCCT	k-d(10)-k-e-k-e-e	79	1169
74785	74801	530021	AAATGCTTAGATTCTCC	e-e-k-d(10)-k-e-k-e	87	1170
74785	74800	530341	AATGCTTAGATTCTCC	e-k-d(10)-k-e-k-e	88	1171
74786	74801	530089	AAATGCTTAGATTCTC	e-k-k-d(10)-k-k-e	71	1172

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74786	74801	530136	AAATGCTTAGATTCTC	e-e-k-d(10)-k-k-e	66	1172
74786	74801	530186	AAATGCTTAGATTCTC	e-d-k-d(10)-k-k-e	51	1172
74786	74801	530236	AAATGCTTAGATTCTC	e-d-d-k-d(9)-k-k-e	74	1172
74786	74801	530286	AAATGCTTAGATTCTC	e-e-e-e-d(9)-k-k-e	56	1172
74869	74884	528870	GTAAGCACCCCTCTGCC	e-e-e-d(10)-k-k-k	26	1173
74871	74886	528871	TTGTAAGCACCCCTCTG	e-e-e-d(10)-k-k-k	14	1174
74873	74888	528872	GGTTGTAAGCACCCCTC	e-e-e-d(10)-k-k-k	47	1175
74874	74889	528873	AGGTTGTAAGCACCCCT	e-e-e-d(10)-k-k-k	40	1176
74875	74890	528874	AAGGTTGTAAGCACCC	e-e-e-d(10)-k-k-k	54	1177
74877	74892	528875	TCAAGGTTGTAAGCAC	e-e-e-d(10)-k-k-k	15	1178
74878	74893	528876	GTCAAGGTTGTAAGCA	e-e-e-d(10)-k-k-k	28	1179
74879	74894	528877	AGTCAAGGTTGTAAGC	e-e-e-d(10)-k-k-k	28	1180
74881	74896	528878	GGAGTCAAGGTTGTAA	e-e-e-d(10)-k-k-k	6	1181
74882	74897	528879	GGGAGTCAAGGTTGTA	e-e-e-d(10)-k-k-k	22	1182
74901	74916	530392	GATCAAGTCCAGGGAG	k-d(10)-k-e-k-e-e	47	1183
74902	74918	530022	CAGATCAAGTCCAGGGA	e-e-k-d(10)-k-e-k-e	80	1184
74902	74917	530342	AGATCAAGTCCAGGGA	e-k-d(10)-k-e-k-e	70	1185
74902	74917	530393	AGATCAAGTCCAGGGA	k-d(10)-k-e-k-e-e	46	1185
74903	74919	530023	GCAGATCAAGTCCAGGG	e-e-k-d(10)-k-e-k-e	74	1186
74903	74918	530090	CAGATCAAGTCCAGGG	e-k-k-d(10)-k-k-e	78	1187
74903	74918	530137	CAGATCAAGTCCAGGG	e-e-k-d(10)-k-k-e	76	1187
74903	74918	530187	CAGATCAAGTCCAGGG	e-d-k-d(10)-k-k-e	68	1187
74903	74918	530237	CAGATCAAGTCCAGGG	e-d-d-k-d(9)-k-k-e	36	1187
74903	74918	530287	CAGATCAAGTCCAGGG	e-e-e-e-d(9)-k-k-e	56	1187
74903	74918	530343	CAGATCAAGTCCAGGG	e-k-d(10)-k-e-k-e	68	1187
74903	74918	530394	CAGATCAAGTCCAGGG	k-d(10)-k-e-k-e-e	49	1187
74904	74919	518343	GCAGATCAAGTCCAGG	e-e-e-d(10)-k-k-k	5	1188
74904	74920	530024	AGCAGATCAAGTCCAGG	e-e-k-d(10)-k-e-k-e	79	1189
74904	74919	530091	GCAGATCAAGTCCAGG	e-k-k-d(10)-k-k-e	81	1188
74904	74919	530138	GCAGATCAAGTCCAGG	e-e-k-d(10)-k-k-e	81	1188
74904	74919	530188	GCAGATCAAGTCCAGG	e-d-k-d(10)-k-k-e	78	1188
74904	74919	530238	GCAGATCAAGTCCAGG	e-d-d-k-d(9)-k-k-e	29	1188
74904	74919	530288	GCAGATCAAGTCCAGG	e-e-e-e-d(9)-k-k-e	69	1188
74904	74919	530344	GCAGATCAAGTCCAGG	e-k-d(10)-k-e-k-e	85	1188
74905	74920	530092	AGCAGATCAAGTCCAG	e-k-k-d(10)-k-k-e	85	1190
74905	74920	530139	AGCAGATCAAGTCCAG	e-e-k-d(10)-k-k-e	79	1190
74905	74920	530189	AGCAGATCAAGTCCAG	e-d-k-d(10)-k-k-e	77	1190
74905	74920	530239	AGCAGATCAAGTCCAG	e-d-d-k-d(9)-k-k-e	61	1190
74905	74920	530289	AGCAGATCAAGTCCAG	e-e-e-e-d(9)-k-k-e	75	1190
74907	74922	528880	ACAGCAGATCAAGTCC	e-e-e-d(10)-k-k-k	65	1191
74908	74923	528881	AACAGCAGATCAAGTC	e-e-e-d(10)-k-k-k	44	1192
74924	74939	528882	ACAACCTAGCCTCTGA	e-e-e-d(10)-k-k-k	39	1193

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74925	74940	528883	AACAACCTAGCCTCTG	e-e-e-d(10)-k-k-k	46	1194
74927	74942	528884	GAAACAACCTAGCCTC	e-e-e-d(10)-k-k-k	37	1195
74928	74943	528885	AGAAACAACCTAGCCT	e-e-e-d(10)-k-k-k	20	1196
74929	74944	528886	CAGAAACAACCTAGCC	e-e-e-d(10)-k-k-k	21	1197
74942	74957	528887	GATAAGGCACCCACAG	e-e-e-d(10)-k-k-k	25	1198
74943	74958	528888	TGATAAGGCACCCACA	e-e-e-d(10)-k-k-k	12	1199
74944	74959	528889	CTGATAAGGCACCCAC	e-e-e-d(10)-k-k-k	25	1200
74946	74961	528890	CCCTGATAAGGCACCC	e-e-e-d(10)-k-k-k	42	1201
74947	74962	528891	GCCCTGATAAGGCACC	e-e-e-d(10)-k-k-k	49	1202
74952	74967	528892	TCCCAGCCCTGATAAG	e-e-e-d(10)-k-k-k	0	1203
74954	74969	528893	TATCCCAGCCCTGATA	e-e-e-d(10)-k-k-k	0	1204
74957	74972	528894	AAGTATCCCAGCCCTG	e-e-e-d(10)-k-k-k	25	1205
74958	74973	528895	GAAGTATCCCAGCCCT	e-e-e-d(10)-k-k-k	39	1206
74959	74974	528896	AGAAGTATCCCAGCCC	e-e-e-d(10)-k-k-k	22	1207
74960	74975	528897	CAGAAGTATCCCAGCC	e-e-e-d(10)-k-k-k	36	1208
75079	75094	528898	TGAGACCAGGATTCCT	e-e-e-d(10)-k-k-k	41	1209
75083	75098	528899	GTCCTGAGACCAGGAT	e-e-e-d(10)-k-k-k	19	1210
75164	75179	528900	AGCTCAACCAGACACG	e-e-e-d(10)-k-k-k	54	311
75166	75181	528901	TGAGCTCAACCAGACA	e-e-e-d(10)-k-k-k	40	1211
75171	75186	528902	TTCCCTGAGCTCAACC	e-e-e-d(10)-k-k-k	32	1212
75179	75194	528903	GAACCATATTCCTGA	e-e-e-d(10)-k-k-k	30	313
75182	75197	528904	TAAGAACCATATTCCT	e-e-e-d(10)-k-k-k	27	1213
75209	75224	518344	GCCACTGGATATCACC	e-e-e-d(10)-k-k-k	89	317
75254	75269	528905	TAAGCCTTTGCCCTGC	e-e-e-d(10)-k-k-k	64	1214
75255	75270	528906	GTAAGCCTTTGCCCTG	e-e-e-d(10)-k-k-k	53	1215
75256	75271	528907	AGTAAGCCTTTGCCCT	e-e-e-d(10)-k-k-k	45	1216
75257	75272	528908	CAGTAAGCCTTTGCCC	e-e-e-d(10)-k-k-k	40	1217
75259	75274	528909	ATCAGTAAGCCTTTGC	e-e-e-d(10)-k-k-k	53	1218
75260	75275	528910	TATCAGTAAGCCTTTG	e-e-e-d(10)-k-k-k	47	1219
75264	75279	528911	AGTTTATCAGTAAGCC	e-e-e-d(10)-k-k-k	58	1220
75270	75285	528912	GACTCAAGTTTATCAG	e-e-e-d(10)-k-k-k	37	1221
75272	75287	528913	CAGACTCAAGTTTATC	e-e-e-d(10)-k-k-k	39	1222
75273	75288	528914	GCAGACTCAAGTTTAT	e-e-e-d(10)-k-k-k	0	1223
75274	75289	528915	GGCAGACTCAAGTTTA	e-e-e-d(10)-k-k-k	1	1224
75275	75290	528916	GGGCAGACTCAAGTTT	e-e-e-d(10)-k-k-k	0	1225
75276	75291	528917	AGGGCAGACTCAAGTT	e-e-e-d(10)-k-k-k	9	1226
75278	75293	528918	CGAGGGCAGACTCAAG	e-e-e-d(10)-k-k-k	2	1227
75280	75295	528919	TACGAGGGCAGACTCA	e-e-e-d(10)-k-k-k	20	324
75281	75296	528920	ATACGAGGGCAGACTC	e-e-e-d(10)-k-k-k	14	1228
75282	75297	528921	CATACGAGGGCAGACT	e-e-e-d(10)-k-k-k	0	1229
75283	75298	528922	TCATACGAGGGCAGAC	e-e-e-d(10)-k-k-k	8	1230
75285	75300	528923	CCTCATACGAGGGCAG	e-e-e-d(10)-k-k-k	2	1231

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75286	75301	528924	CCCTCATACGAGGGCA	e-e-e-d(10)-k-k-k	2	1232
75287	75302	528925	ACCCTCATACGAGGGC	e-e-e-d(10)-k-k-k	0	1233
75412	75427	528926	TACGCACAGGAGAGGC	e-e-e-d(10)-k-k-k	20	1233
75413	75428	528927	ATACGCACAGGAGAGG	e-e-e-d(10)-k-k-k	0	1234
75414	75429	528928	CATACGCACAGGAGAG	e-e-e-d(10)-k-k-k	6	1235
75415	75430	528929	CCATACGCACAGGAGA	e-e-e-d(10)-k-k-k	4	1236
75416	75431	528930	CCCATACGCACAGGAG	e-e-e-d(10)-k-k-k	36	1237
75417	75432	528931	TCCCATACGCACAGGA	e-e-e-d(10)-k-k-k	22	1238
75418	75433	528932	TTCCCATACGCACAGG	e-e-e-d(10)-k-k-k	32	1239
75419	75434	528933	GTTCCCATACGCACAG	e-e-e-d(10)-k-k-k	45	1240
75420	75435	528934	TGTTCCCATACGCACA	e-e-e-d(10)-k-k-k	36	1241
75421	75436	528935	GTGTTCCCATACGCAC	e-e-e-d(10)-k-k-k	20	1242
75421	75436	530395	GTGTTCCCATACGCAC	k-d(10)-k-e-k-e-e	71	1242
75422	75437	528936	GGTGTTCCTACGCA	e-e-e-d(10)-k-k-k	71	1243
75422	75438	530025	AGGTGTTCCTACGCA	e-e-k-d(10)-k-e-k-e	90	1244
75422	75437	530345	GGTGTTCCTACGCA	e-k-d(10)-k-e-k-e	93	1243
75422	75437	530396	GGTGTTCCTACGCA	k-d(10)-k-e-k-e-e	71	1243
75423	75438	528937	AGGTGTTCCTACGC	e-e-e-d(10)-k-k-k	73	1245
75423	75439	530026	TAGGTGTTCCTACGC	e-e-k-d(10)-k-e-k-e	87	1246
75423	75438	530093	AGGTGTTCCTACGC	e-k-k-d(10)-k-k-e	95	1245
75423	75438	530140	AGGTGTTCCTACGC	e-e-k-d(10)-k-k-e	89	1245
75423	75438	530190	AGGTGTTCCTACGC	e-d-k-d(10)-k-k-e	82	1245
75423	75438	530240	AGGTGTTCCTACGC	e-d-d-k-d(9)-k-k-e	50	1245
75423	75438	530290	AGGTGTTCCTACGC	e-e-e-e-d(9)-k-k-e	69	1245
75423	75438	530346	AGGTGTTCCTACGC	e-k-d(10)-k-e-k-e	89	1245
75424	75439	528938	TAGGTGTTCCTACG	e-e-e-d(10)-k-k-k	72	336
75424	75439	530094	TAGGTGTTCCTACG	e-k-k-d(10)-k-k-e	88	336
75424	75439	530141	TAGGTGTTCCTACG	e-e-k-d(10)-k-k-e	80	336
75424	75439	530191	TAGGTGTTCCTACG	e-d-k-d(10)-k-k-e	74	336
75424	75439	530241	TAGGTGTTCCTACG	e-d-d-k-d(9)-k-k-e	53	336
75424	75439	530291	TAGGTGTTCCTACG	e-e-e-e-d(9)-k-k-e	68	336
75425	75440	528939	CTAGGTGTTCCTAC	e-e-e-d(10)-k-k-k	39	1247
75426	75441	528940	GCTAGGTGTTCCTA	e-e-e-d(10)-k-k-k	62	1248
75427	75442	528941	TGCTAGGTGTTCCTA	e-e-e-d(10)-k-k-k	49	1249
75429	75444	528942	CGTGCTAGGTGTTC	e-e-e-d(10)-k-k-k	77	1250
75491	75506	528943	CAAGGTGGTTTTGAGT	e-e-e-d(10)-k-k-k	25	1251
75492	75507	528944	GCAAGGTGGTTTTGAG	e-e-e-d(10)-k-k-k	28	344
75507	75522	528945	CTCTGATCAGCTGAGG	e-e-e-d(10)-k-k-k	74	1252
75508	75523	528946	ACTCTGATCAGCTGAG	e-e-e-d(10)-k-k-k	56	1253
75549	75564	528947	GAGACCAGCTAATTG	e-e-e-d(10)-k-k-k	36	1254
75582	75597	528948	CATCTTAGAGAAGGTC	e-e-e-d(10)-k-k-k	59	1255
75622	75637	528949	TCAACTGTCTCCAGGC	e-e-e-d(10)-k-k-k	67	1256

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75622	75637	530397	TCAACTGTCTCCAGGC	k-d(10)-k-e-k-e-e	60	1256
75623	75638	528950	ATCAACTGTCTCCAGG	e-e-e-d(10)-k-k-k	57	1257
75623	75639	530027	CATCAACTGTCTCCAGG	e-e-k-d(10)-k-e-k-e	56	1258
75623	75638	530347	ATCAACTGTCTCCAGG	e-k-d(10)-k-e-k-e	49	1257
75624	75639	530095	CATCAACTGTCTCCAG	e-k-k-d(10)-k-k-e	40	354
75624	75639	530142	CATCAACTGTCTCCAG	e-e-k-d(10)-k-k-e	43	354
75624	75639	530192	CATCAACTGTCTCCAG	e-d-k-d(10)-k-k-e	42	354
75624	75639	530242	CATCAACTGTCTCCAG	e-d-d-k-d(9)-k-k-e	0	354
75624	75639	530292	CATCAACTGTCTCCAG	e-e-e-e-d(9)-k-k-e	36	354
75624	75639	530398	CATCAACTGTCTCCAG	k-d(10)-k-e-k-e-e	28	354
75625	75641	530028	CACATCAACTGTCTCCA	e-e-k-d(10)-k-e-k-e	57	1259
75625	75640	530348	ACATCAACTGTCTCCA	e-k-d(10)-k-e-k-e	58	1260
75626	75641	530096	CACATCAACTGTCTCC	e-k-k-d(10)-k-k-e	72	356
75626	75641	530143	CACATCAACTGTCTCC	e-e-k-d(10)-k-k-e	74	356
75626	75641	530193	CACATCAACTGTCTCC	e-d-k-d(10)-k-k-e	62	356
75626	75641	530243	CACATCAACTGTCTCC	e-d-d-k-d(9)-k-k-e	34	356
75626	75641	530293	CACATCAACTGTCTCC	e-e-e-e-d(9)-k-k-e	59	356
75628	75643	528951	GACACATCAACTGTCT	e-e-e-d(10)-k-k-k	16	1261
75662	75677	528952	GAAGAGTGTGCTGGA	e-e-e-d(10)-k-k-k	57	1262
75664	75679	528953	CTGAAGAGTGTGCTG	e-e-e-d(10)-k-k-k	46	1263
75666	75681	528954	TACTGAAGAGTGTGTC	e-e-e-d(10)-k-k-k	42	1264
75672	75687	530510	ATTATGTACTGAAGAG	k-d(10)-k-e-k-e-e	53	1265
75673	75688	530504	TATTATGTACTGAAGA	e-k-d(10)-k-e-k-e	25	1266
75673	75688	530511	TATTATGTACTGAAGA	k-d(10)-k-e-k-e-e	31	1266
75674	75689	530432	TTATTATGTACTGAAG	k-d(10)-k-e-k-e-e	15	1267
75674	75689	530463	TTATTATGTACTGAAG	e-k-k-d(10)-k-k-e	20	1267
75674	75689	530472	TTATTATGTACTGAAG	e-e-k-d(10)-k-k-e	17	1267
75674	75689	530480	TTATTATGTACTGAAG	e-d-k-d(10)-k-k-e	4	1267
75674	75689	530488	TTATTATGTACTGAAG	e-d-d-k-d(9)-k-k-e	13	1267
75674	75689	530496	TTATTATGTACTGAAG	e-e-e-e-d(9)-k-k-e	0	1267
75674	75689	530505	TTATTATGTACTGAAG	e-k-d(10)-k-e-k-e	37	1267
75675	75691	530063	GCTTATTATGTACTGAA	e-e-k-d(10)-k-e-k-e	74	1268
75675	75690	530382	CTTATTATGTACTGAA	e-k-d(10)-k-e-k-e	17	1269
75675	75690	530465	CTTATTATGTACTGAA	e-k-k-d(10)-k-k-e	63	1269
75675	75690	530473	CTTATTATGTACTGAA	e-e-k-d(10)-k-k-e	45	1269
75675	75690	530481	CTTATTATGTACTGAA	e-d-k-d(10)-k-k-e	14	1269
75675	75690	530489	CTTATTATGTACTGAA	e-d-d-k-d(9)-k-k-e	13	1269
75675	75690	530497	CTTATTATGTACTGAA	e-e-e-e-d(9)-k-k-e	7	1269
75675	75690	530512	CTTATTATGTACTGAA	k-d(10)-k-e-k-e-e	21	1269
75676	75691	519638	GCTTATTATGTACTGA	e-k-k-d(10)-k-k-e	86	362
75676	75691	530177	GCTTATTATGTACTGA	e-e-k-d(10)-k-k-e	71	362
75676	75691	530227	GCTTATTATGTACTGA	e-d-k-d(10)-k-k-e	51	362

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75676	75691	530277	GCTTATTATGTACTGA	e-d-d-k-d(9)-k-k-e	70	362
75676	75691	530327	GCTTATTATGTACTGA	e-e-e-e-d(9)-k-k-e	61	362
75677	75692	530466	AGCTTATTATGTACTG	e-k-k-d(10)-k-k-e	82	1270
75677	75692	530474	AGCTTATTATGTACTG	e-e-k-d(10)-k-k-e	62	1270
75677	75692	530482	AGCTTATTATGTACTG	e-d-k-d(10)-k-k-e	53	1270
75677	75692	530490	AGCTTATTATGTACTG	e-d-d-k-d(9)-k-k-e	42	1270
75677	75692	530498	AGCTTATTATGTACTG	e-e-e-e-d(9)-k-k-e	45	1270
75677	75692	530506	AGCTTATTATGTACTG	e-k-d(10)-k-e-k-e	70	1270
75678	75693	530467	AAGCTTATTATGTACT	e-k-k-d(10)-k-k-e	50	1271
75678	75693	530475	AAGCTTATTATGTACT	e-e-k-d(10)-k-k-e	26	1271
75678	75693	530483	AAGCTTATTATGTACT	e-d-k-d(10)-k-k-e	19	1271
75678	75693	530491	AAGCTTATTATGTACT	e-d-d-k-d(9)-k-k-e	13	1271
75678	75693	530499	AAGCTTATTATGTACT	e-e-e-e-d(9)-k-k-e	15	1271
75679	75694	528955	TAAGCTTATTATGTAC	e-e-e-d(10)-k-k-k	0	1272
75686	75701	528956	TATCAGTTAAGCTTAT	e-e-e-d(10)-k-k-k	0	1273
75689	75704	528957	GTTTATCAGTTAAGCT	e-e-e-d(10)-k-k-k	31	1274
75726	75741	530433	CAATGGTAAGCCCAAG	k-d(10)-k-e-k-e-e	62	1275
75727	75742	528958	CCAATGGTAAGCCCAA	e-e-e-d(10)-k-k-k	66	1276
75727	75743	530056	CCCAATGGTAAGCCCAA	e-e-k-d(10)-k-e-k-e	73	1277
75727	75742	530383	CCAATGGTAAGCCCAA	e-k-d(10)-k-e-k-e	64	1276
75728	75743	518345	CCCAATGGTAAGCCCA	e-e-e-d(10)-k-k-k	80	366
75728	75743	519636	CCCAATGGTAAGCCCA	e-k-k-d(10)-k-k-e	90	366
75728	75743	530178	CCCAATGGTAAGCCCA	e-e-k-d(10)-k-k-e	86	366
75728	75743	530228	CCCAATGGTAAGCCCA	e-d-k-d(10)-k-k-e	77	366
75728	75743	530278	CCCAATGGTAAGCCCA	e-d-d-k-d(9)-k-k-e	86	366
75728	75743	530328	CCCAATGGTAAGCCCA	e-e-e-e-d(9)-k-k-e	80	366
75729	75744	528959	ACCCAATGGTAAGCCC	e-e-e-d(10)-k-k-k	73	1277
75731	75746	528960	AAACCCAATGGTAAGC	e-e-e-d(10)-k-k-k	43	1278
75732	75747	528961	TAAACCCAATGGTAAG	e-e-e-d(10)-k-k-k	18	1279
75733	75748	528962	TTAAACCCAATGGTAA	e-e-e-d(10)-k-k-k	13	1280
75734	75749	528963	TTTAAACCCAATGGTA	e-e-e-d(10)-k-k-k	2	1281
75741	75756	528964	CCTATGATTTAAACCC	e-e-e-d(10)-k-k-k	17	1282
75745	75760	528965	GGTCCCTATGATTTAA	e-e-e-d(10)-k-k-k	31	1283
75746	75761	528966	AGGTCCCTATGATTTA	e-e-e-d(10)-k-k-k	22	1284
75802	75817	528967	CCTAAGGCCATGAACT	e-e-e-d(10)-k-k-k	19	374
75803	75818	528968	ACCTAAGGCCATGAAC	e-e-e-d(10)-k-k-k	25	1285
75804	75819	528969	TACCTAAGGCCATGAA	e-e-e-d(10)-k-k-k	41	1286
75805	75820	528970	CTACCTAAGGCCATGA	e-e-e-d(10)-k-k-k	55	1287
75806	75821	528971	GCTACCTAAGGCCATG	e-e-e-d(10)-k-k-k	66	1288
75807	75822	528972	TGCTACCTAAGGCCAT	e-e-e-d(10)-k-k-k	56	1289
75808	75823	528973	ATGCTACCTAAGGCCA	e-e-e-d(10)-k-k-k	71	1290
75809	75824	528974	CATGCTACCTAAGGCC	e-e-e-d(10)-k-k-k	58	1291

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75810	75825	528975	ACATGCTACCTAAGGC	e-e-e-d(10)-k-k-k	34	1292
75823	75838	528976	GTTAAGACCAGATACA	e-e-e-d(10)-k-k-k	45	1293
75824	75839	528977	AGTTAAGACCAGATAC	e-e-e-d(10)-k-k-k	40	1294
75825	75840	528978	GAGTTAAGACCAGATA	e-e-e-d(10)-k-k-k	40	1295
75826	75841	528979	AGAGTTAAGACCAGAT	e-e-e-d(10)-k-k-k	62	1296
75831	75846	530399	CAATCAGAGTTAAGAC	k-d(10)-k-e-k-e-e	36	1297
75832	75848	530029	TACAATCAGAGTTAAGA	e-e-k-d(10)-k-e-k-e	29	1298
75832	75847	530349	ACAATCAGAGTTAAGA	e-k-d(10)-k-e-k-e	33	1299
75833	75848	528980	TACAATCAGAGTTAAG	e-e-e-d(10)-k-k-k	0	378
75833	75848	530097	TACAATCAGAGTTAAG	e-k-k-d(10)-k-k-e	41	378
75833	75848	530144	TACAATCAGAGTTAAG	e-e-k-d(10)-k-k-e	16	378
75833	75848	530194	TACAATCAGAGTTAAG	e-d-k-d(10)-k-k-e	28	378
75833	75848	530244	TACAATCAGAGTTAAG	e-d-d-k-d(9)-k-k-e	0	378
75833	75848	530294	TACAATCAGAGTTAAG	e-e-e-e-d(9)-k-k-e	7	378
75835	75850	528981	GCTACAATCAGAGTTA	e-e-e-d(10)-k-k-k	52	1300
75836	75851	528982	TGCTACAATCAGAGTT	e-e-e-d(10)-k-k-k	47	1301
75837	75852	528983	TTGCTACAATCAGAGT	e-e-e-d(10)-k-k-k	44	1302
75849	75864	530400	CTCTCAGAACTTTTGC	k-d(10)-k-e-k-e-e	65	1303
75850	75866	530030	TCCTCTCAGAACTTTTG	e-e-k-d(10)-k-e-k-e	47	1304
75850	75865	530350	CCTCTCAGAACTTTTG	e-k-d(10)-k-e-k-e	54	1305
75851	75866	530098	TCCTCTCAGAACTTTT	e-k-k-d(10)-k-k-e	42	380
75851	75866	530145	TCCTCTCAGAACTTTT	e-e-k-d(10)-k-k-e	38	380
75851	75866	530195	TCCTCTCAGAACTTTT	e-d-k-d(10)-k-k-e	43	380
75851	75866	530245	TCCTCTCAGAACTTTT	e-d-d-k-d(9)-k-k-e	28	380
75851	75866	530295	TCCTCTCAGAACTTTT	e-e-e-e-d(9)-k-k-e	39	380
75957	75972	528984	CCCACGGGATTCCCTC	e-e-e-d(10)-k-k-k	39	1306
75958	75973	528985	ACCCACGGGATTCCCT	e-e-e-d(10)-k-k-k	36	1307
75959	75974	528986	AACCCACGGGATTCCC	e-e-e-d(10)-k-k-k	47	1308
75960	75975	528987	CAACCCACGGGATTCC	e-e-e-d(10)-k-k-k	39	1309
75961	75976	528988	GCAACCCACGGGATTC	e-e-e-d(10)-k-k-k	48	1310
75962	75977	528989	AGCAACCCACGGGATT	e-e-e-d(10)-k-k-k	40	1311
75964	75979	528990	TAAGCAACCCACGGGA	e-e-e-d(10)-k-k-k	27	1312
75965	75980	528991	GTAAGCAACCCACGGG	e-e-e-d(10)-k-k-k	47	1313
75966	75981	528992	GGTAAGCAACCCACGG	e-e-e-d(10)-k-k-k	42	1314
75967	75982	528993	AGGTAAGCAACCCACG	e-e-e-d(10)-k-k-k	54	1315
75967	75982	530434	AGGTAAGCAACCCACG	k-d(10)-k-e-k-e-e	51	1315
75968	75983	528994	TAGGTAAGCAACCCAC	e-e-e-d(10)-k-k-k	53	1316
75968	75984	530064	GTAGGTAAGCAACCCAC	e-e-k-d(10)-k-e-k-e	53	1317
75968	75983	530384	TAGGTAAGCAACCCAC	e-k-d(10)-k-e-k-e	48	1316
75969	75984	528995	GTAGGTAAGCAACCCA	e-e-e-d(10)-k-k-k	64	388
75969	75984	530129	GTAGGTAAGCAACCCA	e-k-k-d(10)-k-k-e	79	388
75969	75984	530179	GTAGGTAAGCAACCCA	e-e-k-d(10)-k-k-e	74	388

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75969	75984	530229	GTAGGTAAGCAACCCA	e-d-k-d(10)-k-k-e	64	388
75969	75984	530279	GTAGGTAAGCAACCCA	e-d-d-k-d(9)-k-k-e	55	388
75969	75984	530329	GTAGGTAAGCAACCCA	e-e-e-e-d(9)-k-k-e	61	388
75971	75986	528996	AGGTAGGTAAGCAACC	e-e-e-d(10)-k-k-k	21	1318
75975	75990	528997	TTATAGGTAGGTAAGC	e-e-e-d(10)-k-k-k	10	1319
75979	75994	528998	CACCTTATAGGTAGGT	e-e-e-d(10)-k-k-k	22	1320
75981	75996	528999	ACCACCTTATAGGTAG	e-e-e-d(10)-k-k-k	15	1321
75984	75999	529000	TAAACCACCTTATAGG	e-e-e-d(10)-k-k-k	0	1322
75985	76000	529001	ATAAACCACCTTATAG	e-e-e-d(10)-k-k-k	7	1323
75997	76012	529002	GGACAGCAGCTTATAA	e-e-e-d(10)-k-k-k	12	1324
75998	76013	529003	AGGACAGCAGCTTATA	e-e-e-d(10)-k-k-k	40	1325
75998	76013	530401	AGGACAGCAGCTTATA	k-d(10)-k-e-k-e-e	41	1325
75999	76014	529004	CAGGACAGCAGCTTAT	e-e-e-d(10)-k-k-k	38	1326
75999	76015	530031	CCAGGACAGCAGCTTAT	e-e-k-d(10)-k-e-k-e	58	1327
75999	76014	530351	CAGGACAGCAGCTTAT	e-k-d(10)-k-e-k-e	58	1326
75999	76014	530402	CAGGACAGCAGCTTAT	k-d(10)-k-e-k-e-e	60	1326
76000	76016	530032	GCCAGGACAGCAGCTTA	e-e-k-d(10)-k-e-k-e	74	1328
76000	76015	530099	CCAGGACAGCAGCTTA	e-k-k-d(10)-k-k-e	73	1329
76000	76015	530146	CCAGGACAGCAGCTTA	e-e-k-d(10)-k-k-e	70	1329
76000	76015	530196	CCAGGACAGCAGCTTA	e-d-k-d(10)-k-k-e	67	1329
76000	76015	530246	CCAGGACAGCAGCTTA	e-d-d-k-d(9)-k-k-e	39	1329
76000	76015	530296	CCAGGACAGCAGCTTA	e-e-e-e-d(9)-k-k-e	67	1329
76000	76015	530352	CCAGGACAGCAGCTTA	e-k-d(10)-k-e-k-e	67	1329
76001	76016	530100	GCCAGGACAGCAGCTT	e-k-k-d(10)-k-k-e	77	1330
76001	76016	530147	GCCAGGACAGCAGCTT	e-e-k-d(10)-k-k-e	84	1330
76001	76016	530197	GCCAGGACAGCAGCTT	e-d-k-d(10)-k-k-e	71	1330
76001	76016	530247	GCCAGGACAGCAGCTT	e-d-d-k-d(9)-k-k-e	53	1330
76001	76016	530297	GCCAGGACAGCAGCTT	e-e-e-e-d(9)-k-k-e	75	1330
76001	76016	530403	GCCAGGACAGCAGCTT	k-d(10)-k-e-k-e-e	77	1330
76002	76018	530033	TGGCCAGGACAGCAGCT	e-e-k-d(10)-k-e-k-e	65	1331
76002	76017	530353	GGCCAGGACAGCAGCT	e-k-d(10)-k-e-k-e	83	1332
76003	76018	530101	TGGCCAGGACAGCAGC	e-k-k-d(10)-k-k-e	59	1333
76003	76018	530148	TGGCCAGGACAGCAGC	e-e-k-d(10)-k-k-e	79	1333
76003	76018	530198	TGGCCAGGACAGCAGC	e-d-k-d(10)-k-k-e	54	1333
76003	76018	530248	TGGCCAGGACAGCAGC	e-d-d-k-d(9)-k-k-e	32	1333
76003	76018	530298	TGGCCAGGACAGCAGC	e-e-e-e-d(9)-k-k-e	73	1333
76014	76029	530404	TTTGAATGCAGTGGCC	k-d(10)-k-e-k-e-e	67	1334
76015	76031	530034	AATTTGAATGCAGTGGC	e-e-k-d(10)-k-e-k-e	69	1335
76015	76030	530354	ATTTGAATGCAGTGGC	e-k-d(10)-k-e-k-e	85	1336
76015	76030	530405	ATTTGAATGCAGTGGC	k-d(10)-k-e-k-e-e	55	1336
76016	76032	530035	GAATTTGAATGCAGTGG	e-e-k-d(10)-k-e-k-e	69	1337
76016	76031	530102	AATTTGAATGCAGTGG	e-k-k-d(10)-k-k-e	71	1338

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76016	76031	530149	AATTTGAATGCAGTGG	e-e-k-d(10)-k-k-e	70	1338
76016	76031	530199	AATTTGAATGCAGTGG	e-d-k-d(10)-k-k-e	58	1338
76016	76031	530249	AATTTGAATGCAGTGG	e-d-d-k-d(9)-k-k-e	47	1338
76016	76031	530299	AATTTGAATGCAGTGG	e-e-e-e-d(9)-k-k-e	47	1338
76016	76031	530355	AATTTGAATGCAGTGG	e-k-d(10)-k-e-k-e	72	1338
76017	76032	530103	GAATTTGAATGCAGTG	e-k-k-d(10)-k-k-e	77	390
76017	76032	530150	GAATTTGAATGCAGTG	e-e-k-d(10)-k-k-e	73	390
76017	76032	530200	GAATTTGAATGCAGTG	e-d-k-d(10)-k-k-e	63	390
76017	76032	530250	GAATTTGAATGCAGTG	e-d-d-k-d(9)-k-k-e	59	390
76017	76032	530300	GAATTTGAATGCAGTG	e-e-e-e-d(9)-k-k-e	65	390
76029	76044	530435	AAGTACACATTGGAAT	k-d(10)-k-e-k-e-e	62	1339
76030	76046	530057	TGAAGTACACATTGGAA	e-e-k-d(10)-k-e-k-e	69	1340
76030	76045	530385	GAAGTACACATTGGAA	e-k-d(10)-k-e-k-e	70	1341
76031	76046	529005	TGAAGTACACATTGGA	e-e-e-d(10)-k-k-k	64	392
76031	76046	530130	TGAAGTACACATTGGA	e-k-k-d(10)-k-k-e	85	392
76031	76046	530180	TGAAGTACACATTGGA	e-e-k-d(10)-k-k-e	82	392
76031	76046	530230	TGAAGTACACATTGGA	e-d-k-d(10)-k-k-e	65	392
76031	76046	530280	TGAAGTACACATTGGA	e-d-d-k-d(9)-k-k-e	75	392
76031	76046	530330	TGAAGTACACATTGGA	e-e-e-e-d(9)-k-k-e	52	392
76039	76054	529006	TTACACTATGAAGTAC	e-e-e-d(10)-k-k-k	16	1342
76116	76131	529007	AGTTAAAGTAGATACA	e-e-e-d(10)-k-k-k	0	1343
76121	76136	529008	CTGGAAGTTAAAGTAG	e-e-e-d(10)-k-k-k	30	397
76130	76145	529009	CGTTTATTTCTGGAAG	e-e-e-d(10)-k-k-k	52	1344
76144	76159	529010	CGGTTTCCTATATAACG	e-e-e-d(10)-k-k-k	21	1345
76145	76160	529011	ACGGTTCCTATATAAC	e-e-e-d(10)-k-k-k	10	1346

Example 14: Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

Gapmers from the study described in Example 13 exhibiting significant *in vitro* inhibition of STAT3 were tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 39.1 nM, 156.3 nM, 625.0 nM, and 2,500.0 nM concentrations of antisense oligonucleotide, as specified in Table 15. After a treatment period of approximately 16 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 15. As illustrated in Table 15, STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

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Table 15
Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

ISIS No	39.1 nM	156.3nM	625.0 nM	2500.0 nM	IC ₅₀ (μ M)
481464	6	51	84	94	0.2
518345	0	9	56	84	0.6
518349	16	3	47	83	0.6
519636	16	41	75	89	0.2
519637	24	43	84	94	0.2
519638	6	34	70	92	0.3
528403	0	4	39	77	0.9
528458	0	15	46	81	0.7
528475	1	10	51	76	0.7
528476	0	11	42	80	0.7
528869	25	19	67	86	0.3
528880	0	3	45	76	0.8
528937	0	1	49	82	0.8
528938	0	9	50	82	0.7
528942	0	20	59	88	0.5
528959	0	4	55	79	0.7
529022	0	0	52	81	0.8
529023	0	0	53	90	0.6
529024	0	0	47	80	0.8
529025	0	11	50	90	0.6
529026	0	31	73	96	0.4
529027	0	7	36	80	0.9
530021	6	30	69	92	0.3
530025	10	33	73	92	0.3
530026	3	18	52	80	0.6
530041	0	28	72	91	0.4
530048	0	22	53	83	0.5
530049	2	16	69	92	0.4
530053	0	16	66	90	0.5
530062	4	56	85	94	0.2
530066	0	12	46	84	0.7
530088	2	39	77	93	0.3
530091	3	12	59	84	0.5
530092	7	27	65	85	0.4
530093	7	46	79	96	0.2
530094	0	17	63	89	0.5
530109	9	30	72	94	0.3
530110	0	23	61	83	0.5

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530112	0	13	42	90	0.6
530114	0	21	62	79	0.6
530116	22	40	71	92	0.2
530123	8	19	72	93	0.3
530130	0	33	64	89	0.4
530131	4	34	81	93	0.3
530135	22	38	79	94	0.2
530138	6	23	57	86	0.4
530140	4	22	62	91	0.4
530147	0	15	51	83	0.6
530156	7	41	81	96	0.2
530161	0	20	46	78	0.7
530170	0	29	67	90	0.4
530175	37	52	84	95	0.1
530178	8	24	70	86	0.4
530180	0	0	61	82	0.6
530181	0	27	52	86	0.5
530185	0	22	54	86	0.5
530190	17	17	60	87	0.4
530206	8	29	73	93	0.3
530225	0	27	67	91	0.4
530228	11	16	64	86	0.4
530261	5	25	57	91	0.4
530270	7	11	62	91	0.4
530275	14	34	73	91	0.3
530278	1	27	60	85	0.4
530285	5	20	61	82	0.5
530306	3	14	66	85	0.5
530311	6	27	59	86	0.4
530320	3	17	56	85	0.5
530325	5	35	70	92	0.3
530328	4	34	61	87	0.4
530340	8	34	74	90	0.3
530341	2	23	77	89	0.4
530344	16	20	64	89	0.4
530345	15	35	77	94	0.2
530346	5	24	66	92	0.4
530353	7	25	57	83	0.5
530354	2	24	60	81	0.5
530359	0	4	44	89	0.7
530361	13	30	59	92	0.3
530365	0	0	45	88	0.7

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530367	0	15	49	88	0.5
530368	0	27	64	89	0.4
530369	10	28	78	95	0.3
530373	13	29	64	92	0.3
530375	0	14	53	90	0.5
530380	8	40	80	94	0.2
530390	11	21	66	90	0.4
530391	20	7	49	86	0.5
530411	5	19	81	95	0.3
530430	0	8	53	91	0.6
530466	0	4	53	87	0.6
530468	4	17	65	90	0.4
530469	8	38	86	94	0.2
530470	5	39	78	91	0.3
530471	0	21	69	91	0.4
530476	7	9	32	89	0.7
530477	0	12	64	87	0.5
530478	0	14	59	90	0.5
530485	0	10	61	85	0.5
530486	0	17	64	80	0.5
530492	0	25	71	89	0.4
530493	4	23	58	88	0.4
530507	5	17	65	82	0.5
530508	0	14	56	89	0.5
530509	0	17	54	86	0.5
530513	6	24	74	91	0.3
530514	1	7	52	78	0.7
530515	0	19	73	89	0.4

Example 15: Antisense inhibition of human STAT3 in HuVEC cells

Additional antisense oligonucleotides were designed targeting a STAT3 nucleic acid and were tested for their effects on STAT3 mRNA *in vitro*. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The chimeric antisense oligonucleotides in Table 16 are 3-10-3 deoxy, MOE and cEt gapmers or 3-10-4 deoxy, MOE and cEt gapmers. The 3-10-3 gapmers are 16 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by

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wings comprising 3 nucleosides each. The 3-10-4 gapmers are 17 nucleosides in length, wherein the central gap segment comprises ten 2'-deoxynucleosides and is flanked on the 5' direction by a wing comprising 3 nucleosides and on the 3' direction by a wing comprising 4 nucleosides. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5'-methylcytosines. The chemistry column of Table 16 presents the sugar motif of each gapmer, where 'e' indicates a 2'-MOE nucleoside, 'k' indicates a constrained ethyl (cEt) nucleoside, and 'd' indicates a 2'-deoxynucleoside.

"Human Target start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Human Target stop site" indicates the 3'-most nucleoside to which the gapmer is targeted in the human gene sequence. Each gapmer listed in Table 16 is targeted to human STAT3 mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_139276.2). Each gapmer listed in Table 17 is targeted to human STAT3 genomic sequence, designated herein as SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_010755.14 truncated from nucleotides 4185000 to 4264000).

Table 16

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

Human Start Site	Human Stop Site	ISIS No	Sequence	Chemistry	% inhibition	SEQ ID NO
730	745	530011	GGAGATTCTCTACCAC	k-k-k-d(10)-e-e-e	73	53
1901	1916	529974	AAGCCCTTGCCAGCCA	e-e-e-d(10)-k-k-k	83	144
1901	1916	530012	AAGCCCTTGCCAGCCA	k-k-k-d(10)-e-e-e	73	144
2206	2221	530015	CCATGATCTTATAGCC	k-k-k-d(10)-e-e-e	38	175
3016	3031	481464	CTATTGATGTCAGC	k-k-k-d(10)-k-k-k	94	245
3461	3476	529975	AGCACCAAGGAGGCTG	e-e-e-d(10)-k-k-k	54	257
3461	3476	530013	AGCACCAAGGAGGCTG	k-k-k-d(10)-e-e-e	58	257
3584	3600	530018	TCCTTAAACCTTCCTAT	e-e-k-d(10)-k-e-k-e	46	1510
3585	3600	529944	TCCTTAAACCTTCCTA	e-e-e-d(10)-k-k-k	44	273
3585	3600	529977	TCCTTAAACCTTCCTA	k-k-k-d(10)-e-e-e	66	273
3592	3608	530019	TTAGATTCTCCTTAAAC	e-e-k-d(10)-k-e-k-e	43	1511
3593	3608	529945	TTAGATTCTCCTTAAA	e-e-e-d(10)-k-k-k	22	1166
3593	3608	529978	TTAGATTCTCCTTAAA	k-k-k-d(10)-e-e-e	49	1166
3596	3612	530020	ATGCTTAGATTCTCCTT	e-e-k-d(10)-k-e-k-e	85	1512
3597	3612	529979	ATGCTTAGATTCTCCT	k-k-k-d(10)-e-e-e	86	1169
3599	3614	529946	AAATGCTTAGATTCTC	e-e-e-d(10)-k-k-k	46	1172
3599	3614	529980	AAATGCTTAGATTCTC	k-k-k-d(10)-e-e-e	25	1172
3716	3731	529947	CAGATCAAGTCCAGGG	e-e-e-d(10)-k-k-k	68	1187
3716	3731	529981	CAGATCAAGTCCAGGG	k-k-k-d(10)-e-e-e	83	1187
3718	3733	529948	AGCAGATCAAGTCCAG	e-e-e-d(10)-k-k-k	75	1190
3718	3733	529982	AGCAGATCAAGTCCAG	k-k-k-d(10)-e-e-e	84	1190

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4236	4251	529983	AGGTGTTCCCATACGC	k-k-k-d(10)-e-e-e	96	1245
4237	4252	529984	TAGGTGTTCCCATACG	k-k-k-d(10)-e-e-e	91	336
4437	4452	529949	CATCAACTGTCTCCAG	e-e-e-d(10)-k-k-k	48	354
4437	4452	529985	CATCAACTGTCTCCAG	k-k-k-d(10)-e-e-e	37	354
4439	4454	529950	CACATCAACTGTCTCC	e-e-e-d(10)-k-k-k	58	356
4439	4454	529986	CACATCAACTGTCTCC	k-k-k-d(10)-e-e-e	72	356
4646	4661	529987	TACAATCAGAGTTAAG	k-k-k-d(10)-e-e-e	0	378
4664	4679	529951	TCCTCTCAGAACTTTT	e-e-e-d(10)-k-k-k	38	380
4664	4679	529988	TCCTCTCAGAACTTTT	k-k-k-d(10)-e-e-e	40	380
4782	4797	530016	GTAGGTAAGCAACCCA	k-k-k-d(10)-e-e-e	60	388
4813	4828	529952	CCAGGACAGCAGCTTA	e-e-e-d(10)-k-k-k	65	1329
4813	4828	529989	CCAGGACAGCAGCTTA	k-k-k-d(10)-e-e-e	63	1329
4814	4829	529953	GCCAGGACAGCAGCTT	e-e-e-d(10)-k-k-k	65	1330
4814	4829	529990	GCCAGGACAGCAGCTT	k-k-k-d(10)-e-e-e	75	1330
4816	4831	529954	TGGCCAGGACAGCAGC	e-e-e-d(10)-k-k-k	79	1333
4816	4831	529991	TGGCCAGGACAGCAGC	k-k-k-d(10)-e-e-e	52	1333
4829	4844	529955	AATTTGAATGCAGTGG	e-e-e-d(10)-k-k-k	52	1338
4829	4844	529992	AATTTGAATGCAGTGG	k-k-k-d(10)-e-e-e	23	1338
4830	4845	529956	GAATTTGAATGCAGTG	e-e-e-d(10)-k-k-k	60	390
4830	4845	529993	GAATTTGAATGCAGTG	k-k-k-d(10)-e-e-e	51	390
4844	4859	530014	TGAAGTACACATTGGA	k-k-k-d(10)-e-e-e	67	392

Table 17

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 2

Human Start Site	Human Stop Site	ISIS No	Sequence	Chemistry	% inhibition	SEQ ID NO
74203	74218	CTATTTGGATGTCAGC	481464	k-k-k-d(10)-k-k-k	94	245
74772	74787	TCCTTAAACCTTCCTA	529944	e-e-e-d(10)-k-k-k	44	273
74780	74795	TTAGATTCTCCTTAAA	529945	e-e-e-d(10)-k-k-k	22	1166
74786	74801	AAATGCTTAGATTCTC	529946	e-e-e-d(10)-k-k-k	46	1172
74903	74918	CAGATCAAGTCCAGGG	529947	e-e-e-d(10)-k-k-k	68	1187
74905	74920	AGCAGATCAAGTCCAG	529948	e-e-e-d(10)-k-k-k	75	1190
75624	75639	CATCAACTGTCTCCAG	529949	e-e-e-d(10)-k-k-k	48	354
75626	75641	CACATCAACTGTCTCC	529950	e-e-e-d(10)-k-k-k	58	356
75851	75866	TCCTCTCAGAACTTTT	529951	e-e-e-d(10)-k-k-k	38	380
76000	76015	CCAGGACAGCAGCTTA	529952	e-e-e-d(10)-k-k-k	65	1329
76001	76016	GCCAGGACAGCAGCTT	529953	e-e-e-d(10)-k-k-k	65	1330
76003	76018	TGGCCAGGACAGCAGC	529954	e-e-e-d(10)-k-k-k	79	1333
76016	76031	AATTTGAATGCAGTGG	529955	e-e-e-d(10)-k-k-k	52	1338
76017	76032	GAATTTGAATGCAGTG	529956	e-e-e-d(10)-k-k-k	60	390
2340	2355	ACATACAGTAAGACCA	529957	e-e-e-d(10)-k-k-k	21	1376
2385	2400	CAAAAATTTACAACCC	529958	e-e-e-d(10)-k-k-k	10	1380

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2410	2425	CCAATGCTTTATCAGC	529959	e-e-e-d(10)-k-k-k	51	1384
2671	2686	AGACTAAAATCAAGGC	529960	e-e-e-d(10)-k-k-k	30	1388
5002	5017	AACTGAAATTCCTTGG	529961	e-e-e-d(10)-k-k-k	52	1395
5701	5716	GTACTCTTTCAGTGGT	529962	e-e-e-d(10)-k-k-k	91	1399
8080	8095	GCAGATTTACCTTCCT	529963	e-e-e-d(10)-k-k-k	55	1409
9125	9140	CTGCCCCCTATGTATAA	529964	e-e-e-d(10)-k-k-k	18	1413
11263	11278	CTGCCCCCTATGTATAA	529964	e-e-e-d(10)-k-k-k	18	1413
9864	9879	GCTTCTTCCTGAGACA	529965	e-e-e-d(10)-k-k-k	52	1417
12347	12362	GCTTCTTCCTGAGACA	529965	e-e-e-d(10)-k-k-k	52	1417
9866	9881	TGGCTTCTTCCTGAGA	529966	e-e-e-d(10)-k-k-k	51	1420
12349	12364	TGGCTTCTTCCTGAGA	529966	e-e-e-d(10)-k-k-k	51	1420
9875	9890	TCCTCCTGTTGGCTTC	529967	e-e-e-d(10)-k-k-k	80	1425
12358	12373	TCCTCCTGTTGGCTTC	529967	e-e-e-d(10)-k-k-k	80	1425
9876	9891	TTCCTCCTGTTGGCTT	529968	e-e-e-d(10)-k-k-k	56	1426
12359	12374	TTCCTCCTGTTGGCTT	529968	e-e-e-d(10)-k-k-k	56	1426
9878	9893	GGTTCCTCCTGTTGGC	529969	e-e-e-d(10)-k-k-k	69	1429
12361	12376	GGTTCCTCCTGTTGGC	529969	e-e-e-d(10)-k-k-k	69	1429
16865	16880	TATAATTGTGTACTGG	529970	e-e-e-d(10)-k-k-k	41	1441
26063	26078	CAACTTTAGCCCCCTTC	529971	e-e-e-d(10)-k-k-k	32	1452
48404	48419	CACACTTCCATTCTA	529972	e-e-e-d(10)-k-k-k	30	1476
71616	71631	CAGTACAATTGCTTCA	529973	e-e-e-d(10)-k-k-k	49	1505
66138	66153	AAGCCCTTGCCAGCCA	529974	e-e-e-d(10)-k-k-k	83	144
74648	74663	AGCACCAAGGAGGCTG	529975	e-e-e-d(10)-k-k-k	54	257
2705	2720	CTAATGGTTCCTTTGTG	529976	e-e-e-d(10)-k-k-k	25	411
74772	74787	TCCTTAAACCTTCCTA	529977	k-k-k-d(10)-e-e-e	66	273
74780	74795	TTAGATTCTCCTTAAA	529978	k-k-k-d(10)-e-e-e	49	1166
74784	74799	ATGCTTAGATTCTCCT	529979	k-k-k-d(10)-e-e-e	86	1169
74786	74801	AAATGCTTAGATTCTC	529980	k-k-k-d(10)-e-e-e	25	1172
74903	74918	CAGATCAAGTCCAGGG	529981	k-k-k-d(10)-e-e-e	83	1187
74905	74920	AGCAGATCAAGTCCAG	529982	k-k-k-d(10)-e-e-e	84	1190
75423	75438	AGGTGTTCCCATACGC	529983	k-k-k-d(10)-e-e-e	96	1245
75424	75439	TAGGTGTTCCCATACG	529984	k-k-k-d(10)-e-e-e	91	336
75624	75639	CATCAACTGTCTCCAG	529985	k-k-k-d(10)-e-e-e	37	354
75626	75641	CACATCAACTGTCTCC	529986	k-k-k-d(10)-e-e-e	72	356
75833	75848	TACAATCAGAGTTAAG	529987	k-k-k-d(10)-e-e-e	0	378
75851	75866	TCCTCTCAGAACTTTT	529988	k-k-k-d(10)-e-e-e	40	380
76000	76015	CCAGGACAGCAGCTTA	529989	k-k-k-d(10)-e-e-e	63	1329
76001	76016	GCCAGGACAGCAGCTT	529990	k-k-k-d(10)-e-e-e	75	1330
76003	76018	TGGCCAGGACAGCAGC	529991	k-k-k-d(10)-e-e-e	52	1333
76016	76031	AATTTGAATGCAGTGG	529992	k-k-k-d(10)-e-e-e	23	1338
76017	76032	GAATTTGAATGCAGTG	529993	k-k-k-d(10)-e-e-e	51	390
2340	2355	ACATACAGTAAGACCA	529994	k-k-k-d(10)-e-e-e	44	1376

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2385	2400	CAAAAATTTACAACCC	529995	k-k-k-d(10)-e-e-e	0	1380
2410	2425	CCAATGCTTTATCAGC	529996	k-k-k-d(10)-e-e-e	65	1384
2671	2686	AGACTAAAATCAAGGC	529997	k-k-k-d(10)-e-e-e	44	1388
5002	5017	AACTGAAATTCCTTGG	529998	k-k-k-d(10)-e-e-e	35	1395
5701	5716	GTACTCTTTCAGTGGT	529999	k-k-k-d(10)-e-e-e	91	1399
8080	8095	GCAGATTTACCTTCCT	530000	k-k-k-d(10)-e-e-e	80	1409
9125	9140	CTGCCCCCTATGTATAA	530001	k-k-k-d(10)-e-e-e	21	1413
11263	11278	CTGCCCCCTATGTATAA	530001	k-k-k-d(10)-e-e-e	21	1413
9864	9879	GCTTCTTCCTGAGACA	530002	k-k-k-d(10)-e-e-e	74	1417
12347	12362	GCTTCTTCCTGAGACA	530002	k-k-k-d(10)-e-e-e	74	1417
9866	9881	TGGCTTCTTCCTGAGA	530003	k-k-k-d(10)-e-e-e	67	1420
12349	12364	TGGCTTCTTCCTGAGA	530003	k-k-k-d(10)-e-e-e	67	1420
9875	9890	TCCTCCTGTTGGCTTC	530004	k-k-k-d(10)-e-e-e	83	1425
12358	12373	TCCTCCTGTTGGCTTC	530004	k-k-k-d(10)-e-e-e	83	1425
9876	9891	TTCCTCCTGTTGGCTT	530005	k-k-k-d(10)-e-e-e	77	1426
12359	12374	TTCCTCCTGTTGGCTT	530005	k-k-k-d(10)-e-e-e	77	1426
9878	9893	GGTTCCTCCTGTTGGC	530006	k-k-k-d(10)-e-e-e	89	1427
12361	12376	GGTTCCTCCTGTTGGC	530006	k-k-k-d(10)-e-e-e	89	1427
16865	16880	TATAATTGTGTACTGG	530007	k-k-k-d(10)-e-e-e	21	1441
26063	26078	CAACTTTAGCCCCCTTC	530008	k-k-k-d(10)-e-e-e	58	1452
48404	48419	CACACTTTCCATTCTA	530009	k-k-k-d(10)-e-e-e	59	1476
71616	71631	CAGTACAATTGCTTCA	530010	k-k-k-d(10)-e-e-e	75	1505
50694	50709	GGAGATTCTCTACCAC	530011	k-k-k-d(10)-e-e-e	73	53
66138	66153	AAGCCCTTGCCAGCCA	530012	k-k-k-d(10)-e-e-e	73	144
74648	74663	AGCACCAAGGAGGCTG	530013	k-k-k-d(10)-e-e-e	58	257
76031	76046	TGAAGTACACATTGGA	530014	k-k-k-d(10)-e-e-e	67	392
67068	67083	CCATGATCTTATAGCC	530015	k-k-k-d(10)-e-e-e	38	175
75969	75984	GTAGGTAAGCAACCCA	530016	k-k-k-d(10)-e-e-e	60	388
2705	2720	CTAATGGTTCTTTGTG	530017	k-k-k-d(10)-e-e-e	46	411
74771	74787	TCCTTAAACCTTCCTAT	530018	e-e-k-d(10)-k-e-k-e	46	1510
74779	74795	TTAGATTCTCCTTAAAC	530019	e-e-k-d(10)-k-e-k-e	43	1511
74783	74799	ATGCTTAGATTCTCCTT	530020	e-e-k-d(10)-k-e-k-e	85	1512

Example 16: Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

Gapmers from the study described in Example 15 exhibiting significant *in vitro* inhibition of STAT3 were tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 39.1 nM, 156.3 nM, 625.0 nM, and 2,500.0 nM concentrations of antisense oligonucleotide, as specified in Table 18. After a treatment period of approximately 16 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human

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STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 18. As illustrated in Table 18, STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 18
Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

ISIS No	39.1 nM	156.3 nM	625.0 nM	2500.0 nM	IC ₅₀ (μM)
481464	41	78	92	91	0.04
529962	30	51	86	95	0.12
529979	0	43	81	95	0.27
529982	0	0	70	90	0.56
529983	31	67	87	94	0.08
529984	17	44	83	97	0.19
529999	29	51	83	96	0.13
530006	18	38	77	94	0.22
530020	2	39	75	92	0.28

Example 17: Effect of ISIS antisense oligonucleotides targeting STAT3 in the treatment of an MDA-MB-231 human breast cancer xenograft model

BALB/c nude mice inoculated with human breast cancer cells MDA-MB-231 were treated with ISIS 481464 and ISIS 481549. ISIS 481549 is cross-reactive with the mouse sequence (i.e, hybridizes to the mouse sequence). Tumor growth and tolerability of oligonucleotides in the mice was evaluated.

Treatment

The study was conducted at Pharmaron Inc (Beijing, P.R. China). The BALB/c nude mice were obtained from Beijing HFK Bio-Technology Co., Ltd. MDA-MB-231 human breast cancer cells were maintained *in vitro* as a monolayer culture in Leibovitz's L-15 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. The cells were maintained at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely sub-cultured twice weekly with trypsin-EDTA treatment. Cells growing at exponential growth phase were harvested and counted for tumor inoculation.

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Three groups of eight randomly assigned 6-8 week-old female BALB/c nude mice each were inoculated in the right flank with the MDA-MB-231 tumor fragments (3 mm x 2 mm x 2 mm, which were generated from tumor inoculation passage) for tumor development. Antisense oligonucleotide treatment started at day 11 after tumor inoculation when the mean tumor size reached approximately 100 mm³. Two of the groups were injected intraperitoneally twice a week for 3 weeks with 25 mg/kg of ISIS 481464 or ISIS 481549. A control group of mice was injected intraperitoneally twice a week for 3 weeks with PBS.

All procedures related to animal handling, care, and treatment, were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC). Animals were routinely checked for any effects of tumor growth on normal behavior, such as mobility, food consumption, body weight changes, and any other abnormal effect.

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis of human STAT3 mRNA levels using primer probe set RTS199, described hereinabove. Murine STAT3 mRNA levels were also measured using primer probe set mSTAT3_LTS00664 (forward sequence CGACAGCTTCCCCATGGA, designated herein as SEQ ID NO: 1513; reverse sequence ATGCCAGTCTTGACTCTCAATC, designated herein as SEQ ID NO: 1514; probe sequence CTGCGGCAGTTCCTGGCACCTT, designated herein as SEQ ID NO: 1515). Results are presented as percent inhibition of STAT3, relative to PBS control, normalized to cyclophilin. As shown in Table 19, treatment with ISIS antisense oligonucleotides resulted in reduction of both human and murine STAT3 mRNA in comparison to the PBS control.

Table 19

Percent inhibition of STAT3 mRNA in the treatment groups relative to the PBS control in the MDA-MB-231 xenograft model

ISIS No	human STAT3	murine STAT3
481464	25	16
481549	22	44

Effect on tumor growth

Tumor size was measured twice weekly in two dimensions using a caliper. Tumor volumes were calculated using the formula: $V = 0.536 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The tumor size was utilized for calculations of the T-C and T_v/C_v values. T-C was calculated with T as the median time (in days) required for the tumors in the treatment groups to reach a pre-determined size (900 mm³), and C as the median time (in days) for the tumors in the control group to reach the same size. The T_v/C_v value (expressed as percentage) is an indication of the anti-tumor effectiveness of the ISIS

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oligonucleotides, where T_V and C_V were the mean volume of the treated and control groups, respectively, on a given day (day 32).

The results are presented in Tables 20 and 21. The data indicates that treatment with ISIS 481464 and ISIS 481549 significantly impeded tumor growth.

5

Table 20

Effect of antisense inhibition of STAT3 on tumor growth in the MDA-MB-231 xenograft model

Day	PBS	ISIS 481464	ISIS 481549
11	103	104	104
15	185	142	158
18	292	200	205
22	519	305	326
25	745	430	436
29	1,332	643	688
32	1,741	921	984

Table 21

Effect of antisense inhibition of STAT3 on tumor growth inhibition in the MDA-MB-231 xenograft model

Treatment	Tumor Size (mm ³) at day 32	T_V/C_V (%)	T-C at 900 mm ³
PBS	1,741	-	-
ISIS 481464	921	53	6
ISIS 481549	984	57	5

10

Body weight measurements

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body weights were measured on a regular basis during the treatment period. The data is presented in Table 22 and indicate that treatment with either ISIS 481464 or ISIS 481549 does not cause significant weight gain or loss.

15

Table 22

Body weight measurements of mice in the MDA-MB-231 xenograft model

	Day 11	Day 15	Day 18	Day 22	Day 25	Day 29	Day 32
PBS	21.8	22.2	22.5	22.5	22.9	23.4	24.0
ISIS 481464	22.3	22.8	23.0	23.2	23.8	23.9	24.9
ISIS 481549	22.2	22.5	23.0	23.3	23.7	23.7	24.6

Example 18: Effect of ISIS antisense oligonucleotides targeting STAT3 in the treatment of an A431 human epidermoid carcinoma xenograft model

20

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BALB/c nude mice inoculated with human epidermoid cancer cells A431 were treated with ISIS 481464 and ISIS 481549. ISIS 481549 is cross-reactive with the mouse sequence (i.e, hybridizes to the mouse sequence). The effect of the treatment on tumor growth and tolerability in the mice was evaluated.

Treatment

5 The study was conducted at Pharmaron Inc (Beijing, P.R. China). The BALB/c nude mice were obtained from Beijing HFK Bio-Technology Co., Ltd. A431 human epidermoid carcinoma cells were maintained *in vitro* as a monolayer culture in DMEM medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The cells were maintained at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely sub-cultured twice
10 weekly with trypsin-EDTA treatment. Cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

Three groups of eight randomly assigned 6-8 week-old female BALB/c nude mice each were inoculated subcutaneously with 5 x 10⁶ A431 tumor cells for tumor development. Antisense oligonucleotide treatment started at day 8 after tumor inoculation when the mean tumor size reached approximately 95 mm³.
15 Two of the groups were injected intraperitoneally twice a week for 4 weeks with 25 mg/kg of ISIS 481464 or ISIS 481549. A control group of mice was injected intraperitoneally twice a week for 4 weeks with PBS.

All procedures related to animal handling, care, and treatment, were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC). At the time of routine monitoring, the animals were checked for any effects of tumor growth on normal behavior, such as mobility,
20 food consumption, body weight changes and any other abnormal effect.

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis of human STAT3 mRNA levels using primer probe set RTS199, described hereinabove. Murine STAT3 mRNA levels were also measured
25 using primer probe set mSTAT3_LTS00664, described hereinabove. Results are presented as percent inhibition of STAT3, relative to PBS control, normalized to cyclophilin. As shown in Table 23, treatment with ISIS antisense oligonucleotides resulted in reduction of both human and murine STAT3 mRNA in comparison to the PBS control.

Table 23

30 Inhibition of STAT3 mRNA in the treatment groups relative to the PBS control in the A431 xenograft model

ISIS No	human STAT3	murine STAT3
481464	63	26

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481549	29	38
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Protein analysis

Protein was extracted from tumor lysates for western analysis of human STAT3 protein levels with STAT3 monoclonal antibody (Cell Signaling Technology, Cat #9135). Results are presented as percent inhibition of STAT3, relative to PBS control, normalized to the house-keeping protein, COX-II. As shown in Table 24, treatment with ISIS antisense oligonucleotides resulted in reduction of STAT3 protein levels in comparison to the PBS control.

Table 24

Inhibition of STAT3 protein levels in the treatment groups relative to the PBS control in the A431 xenograft model

ISIS No	% reduction
481464	99
481549	22

Effect on tumor growth

Tumor size was measured twice weekly in two dimensions using a caliper, and tumor volumes were calculated using the formula: $V = 0.5 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The tumor size was utilized for calculations of the T-C and T_V/C_V values. T-C was calculated with T as the median time (in days) required for the tumors in the treatment groups to reach a pre-determined size (800 mm³), and C as the median time (in days) for the tumors in the control group to reach the same size. The T_V/C_V value (expressed as percentage) is an indication of the anti-tumor effectiveness of the ISIS oligonucleotides, where T_V and C_V were the mean volume of the treated and control groups, respectively, on a given day (day 33).

The results are presented in Tables 25 and 26. The data indicates that treatment with either ISIS 481464 or ISIS 481549 significantly impeded tumor growth.

Table 25

Effect of antisense inhibition of STAT3 on tumor growth in the A431 xenograft model

Days	PBS	ISIS 481464	ISIS 481549
8	94	95	95
14	178	157	132
17	308	261	202
21	528	412	304
24	682	552	426
28	875	698	555
31	1,071	898	716
33	1,210	1,030	858

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Table 26

Effect of antisense inhibition of STAT3 on tumor growth inhibition in the A431 xenograft model

Treatment	Tumor Size (mm ³) at day 33	T _v /C _v (%)	T-C at 800 mm ³
PBS	1,210	-	-
ISIS 481464	1,030	85	3
ISIS 481549	858	71	6

Body weight measurements

5 To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body weights were measured on a regular basis during the treatment period. The data is presented in Table 27 and indicate that treatment with either ISIS 481464 or ISIS 481549 does not affect the overall health of the mice.

Table 27

Body weight measurements of mice in the A431 xenograft model

	Day 8	Day 14	Day 17	Day 21	Day 24	Day 28	Day 31	Day 33
PBS	20	20	20	21	21	21	22	22
ISIS 481464	20	21	21	21	21	22	22	23
ISIS 481549	20	20	21	21	21	22	22	22

10 **Example 19: Effect of ISIS antisense oligonucleotides targeting STAT3 in the treatment of an NCI-H460 human non-small cell lung cancer (NSCLC) xenograft model**

BALB/c nude mice inoculated with human NCI-H460 human NSCLC were treated with ISIS 491464, which targets human STAT3, and ISIS 481549, which targets both human and murine STAT3. The effect of the treatment on tumor growth and tolerability in the mice was evaluated.

Treatment

The study was conducted at Pharmaron Inc (Beijing, P.R. China). The BALB/c nude mice were obtained from Beijing HFK Bio-Technology Co., Ltd. NCI-H460 human NSCLC cells were maintained *in vitro* as a monolayer culture in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The cells were maintained at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely sub-cultured twice weekly with trypsin-EDTA treatment. Cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

25 Three groups of eight randomly assigned 6-8 week-old female BALB/c nude mice each were inoculated subcutaneously with 2 x 10⁶ NCI-H460 tumor cells for tumor development. Antisense oligonucleotide treatment started at day 6 after tumor inoculation when the mean tumor size reached

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approximately 100 mm³. Two of the groups were injected intraperitoneally twice a week for 3 weeks with 25 mg/kg of ISIS 481464 or ISIS 481549. The third group of mice was injected intraperitoneally twice a week for 3 weeks with PBS, and served as the control group.

All procedures related to animal handling, care, and treatment, were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC). At the time of routine monitoring, the animals were checked for any effects of tumor growth on normal behavior, such as mobility, food consumption, body weight changes and any other abnormal effect.

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis of human STAT3 mRNA levels using primer probe set RTS199, described hereinabove. Murine STAT3 mRNA levels were also measured using primer probe set mSTAT3_LTS00664, described hereinabove. Results are presented as percent inhibition of STAT3, relative to PBS control, normalized to cyclophilin. As shown in Table 28, treatment with ISIS antisense oligonucleotides resulted in reduction of both human and murine STAT3 mRNA in comparison to the PBS control.

Table 28

Inhibition of STAT3 mRNA in the treatment groups relative to the PBS control in the NCI-H460 xenograft model

ISIS No	human STAT3	murine STAT3
481464	34	0
481549	20	35

Effect on tumor growth

Tumor size was measured twice weekly in two dimensions using a caliper, and tumor volumes were calculated using the formula: $V = 0.5 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The tumor size was utilized for calculations of the T-C and T_v/C_v values. T-C was calculated with T as the median time (in days) required for the tumors in the treatment groups to reach a pre-determined size (1,500 mm³), and C as the median time (in days) for the tumors in the control group to reach the same size. The T_v/C_v value (expressed as percentage) is an indication of the anti-tumor effectiveness of the ISIS oligonucleotides, where T_v and C_v were the mean volume of the treated and control groups, respectively, on a given day (day 20).

The results are presented in Tables 29 and 30. The data indicates that treatment with either ISIS 481464 or ISIS 481549 significantly impeded tumor growth.

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Table 29

Effect of antisense inhibition of STAT3 on tumor growth in the NCI-H460 xenograft model

Days	PBS	ISIS 481464	ISIS 481549
6	104	104	103
8	303	197	197
11	746	498	443
13	1,175	676	654
15	1,642	982	954
18	2,277	1,571	1,577
20	2,859	1,996	2,093
22	-	2,609	2,679

Table 30

Effect of antisense inhibition of STAT3 on tumor growth inhibition in the NCI-H460 xenograft model

Treatment	Tumor Size (mm ³) at day 20	T _v /C _v (%)	T-C at 1,500 mm ³
PBS	1,210	-	-
ISIS 481464	1,030	85	3
ISIS 481549	858	71	6

Body weight measurements

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body weights were measured on a regular basis during the treatment period. The data is presented in Table 31 and indicate that treatment with either ISIS 481464 or ISIS 481549 does not affect the overall health of the mice.

Table 31

Body weight measurements of mice in the NCI-H460 xenograft model

	Day 6	Day 8	Day 11	Day 13	Day 15	Day 18	Day 20	Day 22
PBS	20	20	20	20	20	20	21	-
ISIS 481464	20	20	20	20	19	19	20	20
ISIS 481549	20	20	20	20	20	19	20	20

Example 20: Effect of antisense inhibition of human STAT3 in a human glioblastoma orthotopic mouse model

NU/J mice orthotopically implanted with human glioblastoma cells were treated with ISIS 455291, a 5-10-5 MOE gapmer having a sequence of CAGCAGATCAAGTCCAGGGA (SEQ ID NO: 1590). The effect of the treatment on tumor growth and tolerability in the mice was evaluated.

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Thirty NU/J mice were stereotactically implanted in the right frontal lobe with 5×10^5 U-87 MG-luc2 cells. On day 15 after tumor cell implantation, 15 of these mice were dosed intracranially with a bolus injection at the site of tumor implantation with 100 μ g of ISIS 455291, which was dissolved in 2 μ L of PBS.

5 The remaining 15 mice were dosed intracranially with a bolus injection at the site of tumor implantation with 2 μ L of PBS. The second group of mice served as the control group.

Analysis

On day 18 after tumor transplantation, five mice from each group were euthanized by CO₂ inhalation and brain samples were collected for RNA analysis. RNA was extracted from tumor tissue for real-time PCR

10 analysis of human STAT3 mRNA levels using primer probe set RTS199, described hereinabove. Treatment with ISIS 455291 resulted in 27% reduction of human STAT3 mRNA in the tumor tissue in comparison to the PBS control.

The remaining mice in each group were monitored regularly up to 2 weeks for survival analysis. The median survival for the PBS control group was 30.5 days. The medial survival for the ISIS oligonucleotide-

15 treated mice was 35 days. The P value was 0.2088.

Example 21: Effect of treatment with ISIS 481549 in APC/Min⁺ mice

The effect of treatment with ISIS 481549 on STAT3 mRNA levels and intestinal adenoma numbers in the APC/Min⁺ mouse model was evaluated. The APC/Min⁺ mice strain is predisposed to spontaneous

20 intestinal adenoma formation throughout the entire intestinal tract at an early age (Moser A.R. et al., Science 1990. 247: 322-324).

Treatment

Two groups of 4 male nine-week-old APC/Min⁺ mice were injected subcutaneously with 5 mg/kg or 25 mg/kg of ISIS 481549 administered five times a week (total weekly doses of 25 mg/kg and 125 mg/kg, respectively) for 4 weeks. A group of 4 male nine-week-old APC/Min⁺ mice were injected subcutaneously

25 with 50 mg/kg of control oligonucleotide, ISIS 141923, administered five times a week (total weekly dose of 250 mg/kg) for 4 weeks. A control group of 4 male nine-week-old APC/Min⁺ mice were injected subcutaneously with PBS administered five times a week for 4 weeks. Mice were euthanized with isoflurane followed by cervical dislocation 48 hrs after the final injection.

30 Colons and intestines were removed, separated from each other and cleaned. Approximately 5 cm of the upper intestinal tract was excised and homogenized in 2.5 mL RLT buffer (Qiagen) with 1% of 2-mercaptoethanol (RLT-BMe) and placed in dry ice. The colon was cut in half and the proximal half of the

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tissue was homogenized in 2.5 mL RLT-BMe and placed in dry ice. A small piece of the liver (0.2 g) was excised and homogenized in RLT-BMe and placed in dry ice.

RNA analysis

RNA was isolated from the tissues using PureLink™ Total RNA Purification kit (Invitrogen; #12173-011A), according to the manufacturer's protocol. RT-PCR was performed using the StepOnePlus system (Applied Biosystems), according to the manufacturer's protocol. Murine primer probe set mSTAT3_LTS000664 (forward primer CGACAGCTTCCCATGGA, designated herein as SEQ ID NO: 1513; reverse primer ATGCCAGTCTTGACTCTCAATC, designated herein as SEQ ID NO: 1514; probe CTGCGGCAGTTCCTGGCACCTT, designated herein as SEQ ID NO: 1515) was used for measuring STAT3 mRNA levels. The mRNA level of the housekeeping gene, Cyclophilin, was measured with the primer probe set mcyclo_24 (forward primer TCGCCGCTTGCTGCA, designated herein as SEQ ID NO: 1516; reverse primer ATCGGCCGTGATGTCGA, designated herein as SEQ ID NO: 1517; probe CCATGGTCAACCCACCGTGTTT, designated herein as SEQ ID NO: 1518) and was used to normalize STAT3 mRNA levels.

Treatment with ISIS 481549 resulted in statistically significant reduction in STAT3 mRNA expression in liver at 25 mg/kg/wk and 125 mg/kg/wk dosing in liver, small intestine and colon (Table 32) compared to the PBS control. Significant differences between the treatment and the control groups were determined using the Student's two-tailed *t* test ($p < 0.05$).

Table 32
Percent inhibition of STAT3 mRNA expression levels in APC/Min+ mice

Treatment (mg/kg/week)	Liver	Small intestine	Colon
ISIS 141923 (250)	0	0	0
ISIS 481549 (125)	98	73	82
ISIS 481549 (25)	79	41	32

Adenoma number analysis

Histological analysis of the small intestine was performed to microscopically evaluate adenoma numbers. Treatment with ISIS 481549 at 125 mg/kg/week resulted in a statistically significant decrease in tumor number compared to the PBS control (Table 33). Significant differences between the treatment and the control groups were determined using the Student's two-tailed *t* test ($p < 0.05$).

Table 33
Adenoma counts in APC/Min+ mice

Treatment (mg/kg/week)	Colon count
ISIS 141923 (250)	5

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ISIS 481549 (125)	1
ISIS 481549 (25)	5
PBS	6

Example 22: Effect of antisense oligonucleotides targeting STAT3 in the treatment of a PC-9 NSCLC xenograft model

BALB/c nude mice (Charles River) inoculated with the human non-small cell lung cancer cell line, PC-9, were treated with ISIS 481549 and ISIS 481464. Tumor growth and STAT3 target reduction in the mice were evaluated.

Treatment

Six- to eight-week old female BALB/c nude mice were inoculated subcutaneously with 7×10^6 PC-9 human NSCLC cells. Mice that displayed a mean tumor volume of 150-200 mm³ were selected and randomized into different treatment groups. Two groups of 7 mice were injected subcutaneously with 25 mg/kg of ISIS 481549 or ISIS 481464 administered five times a week (total weekly doses of 125 mg/kg) for 6 weeks. A group of 7 mice were injected subcutaneously with 25 mg/kg of ISIS 347526 (TCTTATGTTTCCGAACCGTT, no known murine or human target, designated herein as SEQ ID NO: 1519) administered five times a week (total weekly doses of 125 mg/kg) for 6 weeks. A final dose of antisense oligonucleotide was given 24 hrs before the mice were euthanized.

RNA analysis

Tumors were harvested and RNA was isolated using Qiagen RNeasy Mini Kit (#74106), according to the manufacturer's protocol. STAT3 mRNA levels were measured using an ABI StepOnePlus RT-PCR instrument with human STAT3 primer probe set RTS2033 (forward primer GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse primer TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522). The mRNA levels of the housekeeping gene, GAPDH, was measured with the human primer probe set (forward primer GAAGGTGAAGGTCGGAGTC, designated herein as SEQ ID NO: 1523; reverse primer GAAGATGGTGATGGGATTTC, designated herein as SEQ ID NO: 1524; probe CAAGCTTCCCGTTCTCAGCC, designated herein as SEQ ID NO: 1525) and was used to normalize RNA levels. The results are presented in Table 34 and indicate that the antisense oligonucleotides reduced STAT3 mRNA levels.

Table 34

Percent inhibition of STAT3 mRNA expression levels in the NSCLC xenograft model compared to the ASO control

Treatment (mg/kg)	% inhibition
ISIS 481464 (25)	40

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ISIS 481549 (25)	22
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Tumor growth analysis

Tumors were measured regularly throughout the study period. Tumor growth inhibition (TGI) was calculated using the formula

$$TGI = [1 - (X \text{ of STAT3 ASO group (final)} - X \text{ of STAT3 ASO group (day1)}) / (X \text{ of control ASO group (final)} - X \text{ of control ASO group (day1)})] \times 100\%, \text{ where } X = \text{mean tumor volume.}$$

The difference of the treatment group from the control group was evaluated using the ANOVA statistical test. The results are presented in Table 35. The data indicates that tumor growth was significantly inhibited by ISIS 481464 with TGI of 97% by day 52. Treatment by ISIS 481549 inhibited PC-9 tumor growth by 78%.

Table 35
Tumor growth measurements in the NSCLC xenograft model

Day	10	13	18	20	25	28	31	34	38	42	45	48	52
ISIS 481464	233	241	267	240	229	201	201	254	218	222	221	236	255
ISIS 481549	233	217	239	188	237	299	326	318	328	410	341	389	398
ISIS 347526	240	279	295	344	295	354	383	407	540	573	655	890	940

15 *Body weight analysis*

Body weights were measured regularly throughout the study period. The results are presented in Table 36 and indicate that there were no significant changes in body weight of the treatment groups compared to the control groups.

Table 36
Body weight measurements in the NSCLC xenograft model

Day	10	13	18	20	25	28	31	34	38	42	45	48	52
ISIS 481464	18.65	19.44	18.98	19.66	19.40	19.45	19.89	20.26	19.86	20.31	20.13	20.03	20.11
ISIS 481549	18.13	19.06	18.65	19.30	19.31	19.36	19.23	19.18	18.28	17.21	16.49	15.48	15.01
ISIS 347526	18.34	19.29	19.05	19.65	19.63	19.98	20.08	20.69	19.90	20.19	20.25	20.09	20.19

Example 23: Effect of ISIS 481464 in the treatment of an LG-476 NSCLC xenograft model

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice (NSG; JAX #5557), which are immunodeficient, were inoculated with the human non-small cell lung cancer cell line, LG-476 (Jackson Laboratory) and treated with ISIS 481464. Tumor growth and STAT3 target reduction in the mice was evaluated.

Treatment

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Four- to six-week old female NSG mice were inoculated subcutaneously with LG-476 human NSCLC cells and monitored three times weekly for clinical observations, body weights and tumor volume. Once tumors reached 1,000 mm³, the tumors were harvested and fragmented. Tumor fragments measuring 3-5 mm³ were implanted subcutaneously into the right hind flank of 30 NSG mice. The mice were monitored three times a week. When individual tumors reached a volume of 200-250 mm³, the mice were randomly assigned to 2 groups and were injected with 25 mg/kg of ISIS 481464 or PBS administered 5 times a week (weekly doses of 125 mg/kg) for 3 weeks. Tumors were harvested 24 hrs after the last dose.

RNA analysis

Lysates from tumors were prepared using an ABI StepOnePlus RT-PCR instrument with a human-specific primer probe set RTS2033. The mRNA levels of the housekeeping gene, Cyclophilin, was measured with a human-specific primer probe set (forward primer GACGGCGAGCCCTTGG, designated herein as SEQ ID NO: 1526; reverse primer TGCTGTCTTTGGGACCTTGTC, designated herein as SEQ ID NO: 1527; probe CCGCGTCTCCTTTGAGCTGTTTGC, designated herein as SEQ ID NO: 1528). Significant differences between the treatment and the control groups were determined using the Student's two-tailed *t* test (*p*<0.05).

Treatment with ISIS 481464 resulted in 43% reduction of STAT3 mRNA levels in the tumor mass compared to the PBS control (Figure 8), which is statistically significant.

Protein analysis

Total cell lysates were prepared by homogenizing tumor in ice-cold radio-immunoprecipitation assay (RIPA) buffer containing protease inhibitor cocktail. The lysates were analyzed by western blotting using STAT3 antibody (Abcam Antibodies, #ab32500). The house-keeping proteins, cytochrome oxidase II (COXII; #ab79393) and survivin (#ab76424) were also probed. STAT3 levels were normalized to either COXII protein or survivin protein and quantified using ImageJ software.

Treatment with ISIS 481464 resulted in 50% reduction in STAT3 protein levels in the tumor mass compared to the PBS control, which is statistically significant.

Tumor growth analysis

Tumors were measured regularly throughout the study period. Treatment with ISIS 481464 resulted in decrease in tumor volume of approximately 39% compared to the PBS control.

Example 24: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in PC9 cells

ISIS 481464, from the studies described above, was further tested at different doses in PC9 cells, a non small cell lung carcinoma cell line. Cells were plated at a density of 3,000 cells per well. Cells were incubated with 0.02μM, 0.1 μM, 0.5 μM, 2.5 μM, and 10.0 μM concentrations of antisense oligonucleotide,

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as specified in Table 37. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 37. As illustrated in Table 37, ISIS 481464 was able to penetrate the cell membrane.

Table 37

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by PC9 cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	2.5 μ M	10.0 μ M	IC_{50} (μ M)
481464	20	51	84	94	96	0.19

Example 25: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in C42B cells

ISIS 481464, from the studies described above, was further tested at different doses in C42B cells, a prostate cancer cell line. Cells were plated at a density of 3,000 cells per well. Cells were incubated with 0.02 μ M, 0.1 μ M, 0.5 μ M, 2.5 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 38. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

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The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 38. As illustrated in Table 38, ISIS 481464 was able to penetrate the cell membrane.

Table 38

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by C42B cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	2.5 μ M	10.0 μ M	IC_{50} (μ M)
481464	21	38	75	87	96	0.45

Example 26: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in Colo201 cells

ISIS 481464, from the studies described above, was further tested at different doses in Colo201 cells, a colorectal cancer cell line. Cells were plated at a density of 3,000 cells per well. Cells were incubated with 0.02 μ M, 0.1 μ M, 0.5 μ M, 2.5 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 39. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 39. As illustrated in Table 39, ISIS 481464 was able to penetrate the cell membrane.

Table 39

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by Colo201 cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	2.5 μ M	10.0 μ M	IC_{50} (μ M)
481464	36	53	81	93	96	0.09

Example 27: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in BT474M1 cells

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ISIS 481464, from the studies described above, was further tested at different doses in BT474M1 cells, a breast cancer cell line. Cells were plated at a density of 3,000 cells per well. Cells were incubated with 0.02 μ M, 0.1 μ M, 0.5 μ M, 2.5 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 40. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 40. As illustrated in Table 40, ISIS 481464 was able to penetrate the cell membrane.

Table 40

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by BT474M1 cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	2.5 μ M	10.0 μ M	IC_{50} (μ M)
481464	13	25	74	94	95	0.24

Example 28: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in H929 cells

ISIS 481464, from the studies described above, was further tested at different doses in H929 cells, a multiple myeloma cell line. Cells were plated at a density of 10,000-12,000 cells per well. Cells were incubated with 0.01 μ M, 0.5 μ M, 2.5 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 41. After approximately 72 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ

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ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 41. As illustrated in Table 41, ISIS 481464 was able to penetrate the cell membrane.

5

Table 41

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by H929 cells

ISIS No	0.1 μ M	0.5 μ M	2.5 μ M	10.0 μ M	IC ₅₀ (μ M)
481464	91	95	95	95	0.04

Example 29: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in MM1R cells

10

ISIS 481464, from the studies described above, was further tested at different doses in MM1R cells, a multiple myeloma cell line. Cells were plated at a density of 10,000-12,000 cells per well. Cells were incubated with 0.01 μ M, 0.5 μ M, 2.5 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 42. After approximately 72 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

15

20

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 42. As illustrated in Table 42, ISIS 481464 was able to penetrate the cell membrane.

25

Table 42

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by MM1R cells

ISIS No	0.1 μ M	0.5 μ M	2.5 μ M	10.0 μ M	IC ₅₀ (μ M)
481464	91	96	95	95	0.04

BIOL0142WO**Example 30: Effect of antisense oligonucleotides targeting STAT3 in the treatment of an SK-OV3 ovarian cancer xenograft model**

BALB/c nude mice were inoculated with the human ovarian cancer cell line, SK-OV3 and treated with ISIS 481464 or ISIS 481549. ISIS 481549 is cross-reactive with the mouse sequence (i.e., hybridizes to the mouse sequence).

Study 1

Human ovarian cancer SK-OV3 cells (approximately 100mm³) were intraperitoneally injected into nude mice. Ten days later, the mice were inoculated subcutaneously with 25 mg/kg of ISIS 481464 or ISIS 481549, administered twice a week for 11 weeks. The mice were euthanized 24 hrs after the final dose.

RNA analysis

Lysates were prepared by using the RNA extraction kit (Invitrogen) in for RT-PCR analysis of STAT3 mRNA levels, using human primer probe set (RTS2033) and mouse primer probe set (mSTAT3-LTS0664). The results are presented in Table 43. The results are presented as percent inhibition of STAT3, relative to the PBS control. The data indicates that treatment with ISIS antisense oligonucleotides resulted in reduction of both human and murine STAT3 mRNA in comparison to the PBS control.

Table 43

Percent inhibition of STAT3 mRNA in the treatment groups relative to the PBS control in the SK-OV3 xenograft model

ISIS No	human STAT3	murine STAT3
481464	63	0
481549	21	61

Protein analysis

Lysates were prepared with RIPA buffer for western blot analysis of STAT3 protein levels, using an antibody against phosphorylated STAT3 (Cell Signaling). The results are presented in Figure 1. The data indicates that treatment with ISIS 481549 resulted in reduction of phosphorylated STAT3 protein in comparison to the PBS control.

IL-6 level analysis

Lysates were prepared by using the RNA extraction kit (Invitrogen) for RT-PCR analysis of IL-6 mRNA levels, using mouse primer probe set mIL6-LTS00629. The results are presented in Table 44. The results are presented as percent inhibition of IL-6, relative to the PBS control. The data indicates that treatment with ISIS 481549 resulted in significant reduction of both IL-6 mRNA in comparison to the PBS control.

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Table 44

Percent inhibition of IL-6 mRNA in the treatment groups relative to the PBS control in the SK-OV3 xenograft model

ISIS No	Murine IL-6 (%)
481464	8
481549	54

5 *Tumor weight analysis*

Tumors were harvested. Tumor weights were measured and the results are presented in Table 45. The results are presented as percent of the PBS control tumor weight. The data indicates that treatment with ISIS 481549 resulted in significant reduction of tumor weight in comparison to the PBS control.

Table 45

Percent decrease of tumor weight in the treatment groups relative to the PBS control in the SK-OV3 xenograft model

ISIS No	Weight (%)
481464	58
481549	89

10 *Study 2*

Human ovarian cancer SK-OV3 cells (approximately 100mm³) were subcutaneously inoculated into nude mice. Ten days later, the mice were inoculated intraperitoneally with 50 mg/kg of either ISIS 481464 or 50 mg/kg of ISIS 481464 and ISIS 481549 in combination, administered five times a week for 6 weeks. The mice were euthanized 24 hrs after the final dose.

Tumor volume analysis

Tumors were measured regularly using Vernier calipers and tumor volumes were calculated using the formula, tumor volume = $\frac{1}{2}$ (length x width²). The results are presented in Figure 2. The data indicates that treatment of the mice with a combination of ISIS 481464 and ISIS 481549 resulted in significant inhibition of tumor growth.

Example 31: Tolerability study of ISIS 481464 in cynomolgus monkeys

25 The efficacy and tolerability of ISIS 481464 in cynomolgus monkeys was evaluated.

Treatment

Male and female naïve cynomolgus monkeys were assigned to five treatment groups. Three groups of 5 monkeys each received loading doses of 3 mg/kg, 10 mg/kg or 30 mg/kg every two days during the first week of the study (on Days 1, 3, 5 and 7) followed by once weekly administration thereafter (commencing on Day 14). A control group of 5 monkeys received PBS every two days during the first week of the study (on Days 1, 3, 5 and 7) as the loading dose, followed by once weekly administration thereafter (commencing on

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Day 14). These doses were administered via a one-hour intravenous (i.v.) infusion. A fifth group of 5 monkeys received loading doses of 30 mg/kg administered subcutaneously every two days during the first week of the study (on Days 1, 3, 5 and 7) followed by once weekly subcutaneous (s.c.) administration thereafter (commencing on Day 14).

5 For the i.v. infusions, the animals were restrained, without sedation, to a chair restraint. A catheter was placed in one of the cephalic veins and ISIS 481464 solution at the appropriate dose was infused at a constant rate over approximately 1 hour using a calibrated syringe pump (Stoelting Co, USA). The dosing site was rotated between right and left arms and the dosing time was recorded. The infusion rate was selected to deliver the calculated dose volume and the accuracy of the pumps was monitored and recorded for each
10 dose. At the end of infusion period, the dosing solution was switched to PBS. In case of s.c. administration, the injections were performed in clock-wise rotation at 4 sites on the back. Injection sites were maintained by periodic shaving and permanently numbered by tattooing.

Three monkeys from each group were sacrificed on day 44, which was approximately 48 hrs following the last dose on day 42. The other 2 monkeys from each group are being observed for toxicological
15 effects. Scheduled euthanasia of the animals was conducted by exsanguination after ketamine/xylazine-induced anesthesia and administration of sodium pentobarbital. The protocols described in the Example were approved by the Institutional Animal Care and Use Committee (IACUC).

RNA analysis

Liver tissue was homogenized in 3 mL of RLT lysis buffer (Qiagen) supplemented with 1% of 2-
20 mercaptoethanol (Sigma). RNA was purified from the resulting homogenate using Qiagen RNeasy 96-well plate for RNA purification, according to the manufacturer's protocol. After purification, the RNA samples were subjected to RT-PCR analysis using Perkin-Elmer ABI Prism 7700 Sequence Detection System and STAT3 primer probe set RTS3235 (forward primer AAGTTTATCTGTGTGACACCAACGA, designated herein as SEQ ID NO: 1532; reverse primer CTTCAACCATTATTTCCAAACTGCAT, designated herein as
25 SEQ ID NO: 1533; probe TGCCGATGTCCCCCGCA, designated herein as SEQ ID NO: 1534). STAT3 mRNA levels were normalized to monkey CyclophilinA, which was quantitated using primer probe set mk_cycloA_2nd (forward primer TGCTGGACCCAACACAAATG, designated herein as SEQ ID NO: 1535; reverse primer TGCCATCCAACCACTCAGTC, designated herein as SEQ ID NO: 1536; probe TTCCCAGTTTTTCATCTGCACTGCCAX, designated herein as SEQ ID NO: 1537).

30 Treatment with ISIS 481464 at 30 mg/kg dose concentrations either via i.v. infusion or s.c. injection resulted in statistically significant reduction in STAT3 mRNA expression in liver (Table 46) compared to the PBS control. Significant differences between the treatment and the control groups were determined using the Student's *t* test ($p < 0.05$).

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Table 46
Percent inhibition of STAT3 mRNA levels in cynomolgus monkeys

Treatment	% inhibition
3 mg i.v.	0
10 mg i.v.	7
30 mg i.v.	52
30 mg s.c.	51

Protein analysis

Liver tissue was homogenized in 1 mL of ice-cold RIPA buffer (Sigma) containing inhibitor cocktails of both proteases and phosphatases (Roche). Total lysates were separated by Bis-Tris PAGE (Invitrogen), transferred to a PVDF membrane, and immunoblotted using primary antibodies for STAT3 (Cell Signaling, #9132) and GAPDH (Advanced Immunochemicals, #06-1-G4-C5). Immunospecific bands were detected with the Enhanced Chemiluminescence Plus detection kit (Amersham Biosciences) after exposure to X-ray film. The intensity of the bands was then scanned and quantified using ImageJ software. Significant differences between the treatment and the control groups were determined using the Student's *t* test ($p < 0.05$).

There was a dose-dependent decrease in STAT3 protein levels, as shown in Table 47, with 33% and 82% reduction at 3 mg/kg/week and 10 mg/kg/week respectively. STAT3 protein was undetectable at 30 mg/kg/week irrespective of the dosing route.

Table 47
Percent inhibition of STAT3 protein levels in cynomolgus monkeys

Treatment	% inhibition
3 mg i.v.	33
10 mg i.v.	82
30 mg i.v.	100
30 mg s.c.	100

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, blood samples were collected from all the study groups. The blood samples were collected via femoral venipuncture on day 44, 48 hrs post-dosing. Blood samples (1mL) were collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for approximately 60 min and then centrifuged at 3,000 rpm for 10 min to obtain serum. Levels of various liver function markers were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Plasma levels of ALT and AST were measured and the results are

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presented in Table 48, expressed in IU/L. Male and female monkey data is presented separately. The results indicate that treatment with ISIS 481464 had no effect on liver function outside the expected range for antisense oligonucleotides.

Table 48

Effect of antisense oligonucleotide treatment on liver function markers in cynomolgus monkey plasma

	Male ALT (IU/L)	Female ALT (IU/L)	Male AST (IU/L)	Female AST (IU/L)
PBS	59	69	83	69
3 mg/kg i.v.	47	56	50	47
10 mg/kg i.v.	56	89	70	60
30 mg/kg i.v.	74	75	60	73
30 mg/kg s.c.	62	78	61	92

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, blood samples were collected from all the study groups. The blood samples were collected via femoral venipuncture on day 44, 48 hrs post-dosing. Blood samples (1mL) were collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for approximately 60 min and then centrifuged at 3,000 rpm for 10 min to obtain serum. Levels of various kidney function markers were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in Table 49, expressed in mg/dL. The plasma chemistry data indicate that treatment with ISIS 481464 did not have any effect on the kidney function outside the expected range for antisense oligonucleotides.

Table 49

Effect of antisense oligonucleotide treatment on plasma BUN and creatinine levels (mg/dL) in cynomolgus monkeys

	Male BUN	Female BUN	Male Creatinine	Female Creatinine
PBS	19	30	0.68	0.88
3 mg/kg i.v.	23	28	0.85	0.86
10 mg/kg i.v.	26	27	0.89	0.94
30 mg/kg i.v.	25	26	0.91	0.86
30 mg/kg s.c.	27	28	0.97	0.85

Body weight measurements

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body weights were measured and are presented in Tables 50 and 51. The results indicate that effect of treatment with ISIS 481464 on body weights was within the expected range for antisense oligonucleotides.

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Table 50

Effect of antisense oligonucleotide treatment on body weights (g) in male cynomolgus monkeys

	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
PBS	2523	2463	2484	2471	2509	2523	2551
3 mg/kg i.v.	2604	2564	2594	2572	2589	2654	2687
10 mg/kg i.v.	2603	2453	2581	2561	2591	2633	2655
30 mg/kg i.v.	2608	2583	2613	2644	2668	2713	2776
30 mg/kg s.c.	2533	2441	2470	2521	2554	2609	2619

Table 51

Effect of antisense oligonucleotide treatment on body weights (g) in female cynomolgus monkeys

	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
PBS	2266	2252	2276	2237	2362	2365	2373
3 mg/kg i.v.	2253	2242	2283	2250	2346	2350	2377
10 mg/kg i.v.	2293	2277	2318	2254	2358	2387	2361
30 mg/kg i.v.	2259	2261	2289	2268	2368	2412	2406
30 mg/kg s.c.	2293	2275	2322	2281	2385	2389	2394

Example 32: Antisense inhibition of human STAT3 in HuVEC cells

Antisense oligonucleotides were designed targeting a STAT3 nucleic acid and were tested for their effects on STAT3 mRNA in vitro. Cultured HuVEC cells at a density of 5,000 cells per well were transfected using LipofectAMINE 2000® reagent with 30 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS2033 (forward sequence GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 5; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 6; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 7) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The chimeric antisense oligonucleotides in Tables 52 and 53 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5'-methylcytosines. "Human Target start site"

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indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Human Target stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Table 52 is targeted to human STAT3 mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_139276.2). Each gapmer listed in Table 53 is targeted to human STAT3 genomic sequence, designated herein as SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_010755.14 truncated from nucleotides 4185000 to 4264000).

The potency of the gapmers was compared to ISIS 337332, ISIS 337333, and ISIS 345785, which are also 5-10-5 MOE gapmers targeting human STAT3, and which are further described in USPN 7,307,069, incorporated herein by reference.

Table 52

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1

ISIS NO	Human Start Site	Human Stop Site	Sequence	% inhibition	SEQ ID NO
337332	1898	1917	GAAGCCCTTGCCAGCCATGT	91	1541
337333	1903	1922	AAGGAGAAGCCCTTGCCAGC	87	1542
345785	2267	2286	TGCCTCCTCCTTGGAATGT	82	1543
455860	2831	2850	ACACAAGACATTCCTTTTT	64	1544
455246	3452	3471	CAAGGAGGCTGTAACTGAA	84	1545
455247	3454	3473	ACCAAGGAGGCTGTAACTG	78	1546
455248	3456	3475	GCACCAAGGAGGCTGTAAAC	69	1547
455249	3458	3477	AAGCACCAAGGAGGCTGTAA	83	1548
455250	3460	3479	TAAAGCACCAAGGAGGCTGT	77	1549
455251	3462	3481	CTTAAAGCACCAAGGAGGCT	78	1550
455252	3464	3483	TGCTTAAAGCACCAAGGAGG	80	1551
455253	3466	3485	AATGCTTAAAGCACCAAGGA	75	1552
455254	3468	3487	TGAATGCTTAAAGCACCAAG	80	1553
455255	3470	3489	GCTGAATGCTTAAAGCACCA	82	1554
455256	3472	3491	AAGCTGAATGCTTAAAGCAC	67	1555
455257	3474	3493	GGAAGCTGAATGCTTAAAGC	79	1556
455258	3476	3495	AAGGAAGCTGAATGCTTAAA	79	1557
455259	3478	3497	TGAAGGAAGCTGAATGCTTA	72	1558
455260	3480	3499	CCTGAAGGAAGCTGAATGCT	75	1559
455261	3527	3546	TAAGGGTTTGACCTGAAGCC	72	1560
455262	3577	3596	TAAACCTTCCTATTTCAACA	77	1561
455263	3579	3598	CTTAAACCTTCCTATTTCAA	64	1562
455264	3581	3600	TCCTTAAACCTTCCTATTTC	73	1563
455265	3583	3602	TCTCCTTAAACCTTCCTATT	87	1564
455266	3585	3604	ATTCTCCTTAAACCTTCCTA	80	1565
455267	3587	3606	AGATTCTCCTTAAACCTTCC	87	1566

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455268	3589	3608	TTAGATTCTCCTTAAACCTT	84	1567
455269	3591	3610	GCTTAGATTCTCCTTAAACC	87	1568
455270	3593	3612	ATGCTTAGATTCTCCTTAAA	87	1569
455271	3595	3614	AAATGCTTAGATTCTCCTTA	89	1570
455272	3597	3616	TAAAATGCTTAGATTCTCCT	88	1571
455273	3639	3658	ATACATTACAAAGGAAAATA	12	1572
455274	3641	3660	CAATACATTACAAAGGAAAA	28	1573
455275	3673	3692	CACCCTCTGCCCAGCCTTAC	63	1574
455276	3675	3694	AGCACCTCTGCCCAGCCTT	79	1575
455277	3677	3696	TAAGCACCTCTGCCCAGCC	65	1576
455278	3679	3698	TGTAAGCACCTCTGCCCAG	62	1577
455279	3681	3700	GTTGTAAGCACCTCTGCCC	62	1578
455280	3683	3702	AGGTTGTAAGCACCTCTGC	75	1579
455281	3685	3704	CAAGTTGTAAGCACCTCT	83	1580
455282	3687	3706	GTCAAGTTGTAAGCACCT	86	1581
455283	3689	3708	GAGTCAAGTTGTAAGCACC	69	1582
455284	3691	3710	GGGAGTCAAGTTGTAAGCA	37	1583
455285	3693	3712	AAGGGAGTCAAGTTGTAAG	56	1584
455286	3695	3714	GAAAGGGAGTCAAGTTGTA	61	1585
455287	3697	3716	GAGAAAGGGAGTCAAGTTG	56	1586
455288	3709	3728	ATCAAGTCCAGGGAGAAAGG	55	1587
455289	3711	3730	AGATCAAGTCCAGGGAGAAA	69	1588
455290	3713	3732	GCAGATCAAGTCCAGGGAGA	80	1589
455291	3715	3734	CAGCAGATCAAGTCCAGGGA	90	1590
455292	3717	3736	AACAGCAGATCAAGTCCAGG	77	1591
455293	3719	3738	GAAACAGCAGATCAAGTCCA	81	1592
455294	3721	3740	CTGAAACAGCAGATCAAGTC	75	1593
455295	3723	3742	CTCTGAAACAGCAGATCAAG	76	1594
455296	3725	3744	GCCTCTGAAACAGCAGATCA	74	1595
455297	3727	3746	TAGCCTCTGAAACAGCAGAT	75	1596
455298	3729	3748	CCTAGCCTCTGAAACAGCAG	76	1597
455299	3731	3750	AACCTAGCCTCTGAAACAGC	83	1598
455300	3733	3752	ACAACCTAGCCTCTGAAACA	57	1599
455301	3735	3754	AAACAACCTAGCCTCTGAAA	72	1600
455302	3737	3756	AGAAACAACCTAGCCTCTGA	78	1601
455303	3739	3758	ACAGAAACAACCTAGCCTCT	69	1602
455304	3741	3760	CCACAGAAACAACCTAGCCT	70	1603
455305	3743	3762	ACCCACAGAAACAACCTAGC	80	1604
455306	3745	3764	GCACCCACAGAAACAACCTA	70	1605
455307	3747	3766	AGGCACCCACAGAAACAACC	75	1606
455308	3749	3768	TAAGGCACCCACAGAAACAA	70	1607
455309	3751	3770	GATAAGGCACCCACAGAAAC	65	1608

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455310	3753	3772	CTGATAAGGCACCCACAGAA	66	1609
455311	3755	3774	CCCTGATAAGGCACCCACAG	81	1610
455312	3757	3776	AGCCCTGATAAGGCACCCAC	79	1611
455313	3759	3778	CCAGCCCTGATAAGGCACCC	74	1612
455314	3761	3780	TCCCAGCCCTGATAAGGCAC	74	1613
455315	3763	3782	TATCCCAGCCCTGATAAGGC	66	1614
455316	3765	3784	AGTATCCCAGCCCTGATAAG	48	1615
455317	3767	3786	GAAGTATCCCAGCCCTGATA	63	1616
455318	3769	3788	CAGAAGTATCCCAGCCCTGA	82	1617
455319	3771	3790	ATCAGAAGTATCCCAGCCCT	80	1618
455320	3879	3898	GATTCCTAAAACAAACAGGA	37	1619
455321	3881	3900	AGGATTCCTAAAACAAACAG	42	1620
455322	3883	3902	CCAGGATTCCTAAAACAAAC	72	1621
455323	3885	3904	GACCAGGATTCCTAAAACAA	71	1622
455324	3887	3906	GAGACCAGGATTCCTAAAAC	43	1623
455325	3889	3908	CTGAGACCAGGATTCCTAAA	77	1624
455326	3891	3910	TCCTGAGACCAGGATTCCTA	76	1625
455327	3893	3912	GGTCCTGAGACCAGGATTCC	69	1626
455328	3895	3914	GAGGTCCTGAGACCAGGATT	76	1627
455329	3897	3916	ATGAGGTCCTGAGACCAGGA	81	1628
455330	3899	3918	CCATGAGGTCCTGAGACCAG	84	1629
455331	3901	3920	TTCCATGAGGTCCTGAGACC	75	1630
455332	3903	3922	TCTTCCATGAGGTCCTGAGA	75	1631
455333	3905	3924	CTTCTTCCATGAGGTCCTGA	79	1632
455334	3907	3926	CTCTTCTTCCATGAGGTCCT	83	1633
455335	3909	3928	CCCTCTTCTTCCATGAGGTC	74	1634
455336	3911	3930	CCCCCTCTTCTTCCATGAGG	72	1635
455337	3913	3932	CTCCCCCTCTTCTTCCATGA	72	1636
455338	3977	3996	CCTGAGCTCAACCAGACACG	79	1637
455339	3979	3998	TCCCTGAGCTCAACCAGACA	73	1638
455340	3981	4000	ATTCCTGAGCTCAACCAGA	75	1639
455341	3983	4002	ATATTCCCTGAGCTCAACCA	65	1640
455342	3985	4004	CCATATTCCCTGAGCTCAAC	78	1641
455343	3987	4006	AACCATATTCCCTGAGCTCA	81	1642
455344	3989	4008	AGAACCATATTCCCTGAGCT	77	1643
455345	3991	4010	TAAGAACCATATTCCCTGAG	73	1644
455346	3993	4012	GCTAAGAACCATATTCCCTG	81	1645
455347	4067	4086	TCAGTAAGCCTTTGCCCTGC	79	1646
455348	4069	4088	TATCAGTAAGCCTTTGCCCT	72	1647
455349	4071	4090	TTTATCAGTAAGCCTTTGCC	76	1648
455350	4073	4092	AGTTTATCAGTAAGCCTTTG	84	1649
455351	4075	4094	CAAGTTTATCAGTAAGCCTT	82	1650

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455352	4077	4096	CTCAAGTTTATCAGTAAGCC	82	1651
455353	4079	4098	GACTCAAGTTTATCAGTAAG	70	1652
455354	4081	4100	CAGACTCAAGTTTATCAGTA	78	1653
455355	4083	4102	GGCAGACTCAAGTTTATCAG	67	1654
455356	4085	4104	AGGGCAGACTCAAGTTTATC	51	1655
455357	4087	4106	CGAGGGCAGACTCAAGTTTA	54	1656
455358	4089	4108	TACGAGGGCAGACTCAAGTT	56	1657
455359	4091	4110	CATACGAGGGCAGACTCAAG	59	1658
455360	4093	4112	CTCATACGAGGGCAGACTCA	74	1659
455361	4095	4114	CCCTCATACGAGGGCAGACT	67	1660
455362	4122	4141	CAGCCTCAGAGGGAGGCCAG	40	1661
455363	4124	4143	ACCAGCCTCAGAGGGAGGCC	34	1662
455364	4126	4145	TCACCAGCCTCAGAGGGAGG	49	1663
455365	4128	4147	AGTCACCAGCCTCAGAGGGA	50	1664
455366	4225	4244	CCCATACGCACAGGAGAGGC	81	1665
455367	4227	4246	TTCCCATACGCACAGGAGAG	72	1666
455368	4229	4248	TGTTCCCATACGCACAGGAG	80	1667
455369	4231	4250	GGTGTTCCTACGCACAGG	76	1668
455370	4233	4252	TAGGTGTTCCTACGCACA	87	1669
455371	4235	4254	GCTAGGTGTTCCTACGCA	92	1670
455372	4237	4256	GTGCTAGGTGTTCCTACG	81	1671
455373	4304	4323	GAGGCAAGGTGGTTTTGAGT	55	1672
455374	4306	4325	CTGAGGCAAGGTGGTTTTGA	74	1673
455375	4308	4327	AGCTGAGGCAAGGTGGTTTT	79	1674
455376	4310	4329	TCAGCTGAGGCAAGGTGGTT	80	1675
455377	4312	4331	GATCAGCTGAGGCAAGGTGG	77	1676
455378	4314	4333	CTGATCAGCTGAGGCAAGGT	60	1677
455379	4316	4335	CTCTGATCAGCTGAGGCAAG	74	1678
455380	4318	4337	AACTCTGATCAGCTGAGGCA	77	1679
455381	4320	4339	GAAACTCTGATCAGCTGAGG	78	1680
455382	4322	4341	CAGAAACTCTGATCAGCTGA	78	1681
455383	4360	4379	CAGAGACCAGCTAATTTGAT	69	1682
455384	4362	4381	TTCAGAGACCAGCTAATTTG	78	1683
455385	4364	4383	AATTCAGAGACCAGCTAATT	77	1684
455386	4366	4385	TTAATTCAGAGACCAGCTAA	83	1685
455387	4423	4442	CTCCAGGCAGGAGGACTGGG	79	1686
455388	4425	4444	GTCTCCAGGCAGGAGGACTG	65	1687
455389	4427	4446	CTGTCTCCAGGCAGGAGGAC	57	1688
455390	4429	4448	AACTGTCTCCAGGCAGGAGG	75	1689
455391	4431	4450	TCAACTGTCTCCAGGCAGGA	86	1690
455392	4433	4452	CATCAACTGTCTCCAGGCAG	80	1691
455393	4435	4454	CACATCAACTGTCTCCAGGC	86	1692

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455394	4437	4456	GACACATCAACTGTCTCCAG	85	1693
455395	4471	4490	GAAGAGTGTGCTGGAGAAG	73	1694
455396	4473	4492	CTGAAGAGTGTGCTGGAGA	78	1695
455397	4475	4494	TACTGAAGAGTGTGCTGGA	83	1696
455398	4477	4496	TGTACTGAAGAGTGTGCTG	86	1697
455399	4479	4498	TATGTACTGAAGAGTGTGTC	74	1698
455400	4481	4500	ATTATGTACTGAAGAGTGTT	74	1699
455401	4483	4502	TTATTATGTACTGAAGAGTG	84	1700
455402	4485	4504	GCTTATTATGTACTGAAGAG	84	1701
455403	4487	4506	AAGCTTATTATGTACTGAAG	77	1702
455404	4489	4508	TTAAGCTTATTATGTACTGA	75	1703
455405	4491	4510	AGTTAAGCTTATTATGTACT	81	1704
455406	4493	4512	TCAGTTAAGCTTATTATGTA	58	1705
455407	4495	4514	TATCAGTTAAGCTTATTATG	65	1706
455408	4497	4516	TTTATCAGTTAAGCTTATTA	46	1707
455409	4499	4518	TGTTTATCAGTTAAGCTTAT	68	1708
455410	4501	4520	TCTGTTTATCAGTTAAGCTT	83	1709
455411	4539	4558	AACCCAATGGTAAGCCCAAG	87	1710
455412	4541	4560	TAAACCCAATGGTAAGCCCA	87	1711
455413	4543	4562	TTTAAACCCAATGGTAAGCC	78	1712
455414	4545	4564	GATTTAAACCCAATGGTAAG	31	1713
455415	4547	4566	ATGATTTAAACCCAATGGTA	71	1714
455416	4549	4568	CTATGATTTAAACCCAATGG	67	1715
455417	4551	4570	CCCTATGATTTAAACCCAAT	70	1716
455418	4553	4572	GTCCCTATGATTTAAACCCA	83	1717
455419	4555	4574	AGGTCCCTATGATTTAAACC	64	1718
455420	4589	4608	TATCTGCTCCAGAGAAGCCC	76	1719
455421	4591	4610	AATATCTGCTCCAGAGAAGC	78	1720
455422	4614	4633	CTACCTAAGGCCATGAACTT	74	1721
455423	4616	4635	TGCTACCTAAGGCCATGAAC	82	1722
455424	4618	4637	CATGCTACCTAAGGCCATGA	84	1723
455425	4636	4655	CAGAGTTAAGACCAGATACA	84	1724
455426	4638	4657	ATCAGAGTTAAGACCAGATA	83	1725
455427	4640	4659	CAATCAGAGTTAAGACCAGA	77	1726
455428	4642	4661	TACAATCAGAGTTAAGACCA	81	1727
455429	4644	4663	GCTACAATCAGAGTTAAGAC	86	1728
455430	4646	4665	TTGCTACAATCAGAGTTAAG	85	1729
455431	4648	4667	TTTTGCTACAATCAGAGTTA	85	1730
455432	4650	4669	ACTTTTGCTACAATCAGAGT	73	1731
455433	4652	4671	GAACTTTGCTACAATCAGA	80	1732
455434	4654	4673	CAGAACTTTTGCTACAATCA	82	1733
455435	4656	4675	CTCAGAACTTTTGCTACAAT	79	1734

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455436	4658	4677	CTCTCAGAACTTTTGCTACA	76	1735
455437	4660	4679	TCCTCTCAGAACTTTTGCTA	75	1736
455438	4662	4681	GCTCCTCTCAGAACTTTTGC	85	1737
455439	4664	4683	CAGCTCCTCTCAGAACTTTT	85	1738
455440	4666	4685	CTCAGCTCCTCTCAGAACTT	80	1739
455441	4668	4687	GGCTCAGCTCCTCTCAGAAC	75	1740
455442	4770	4789	GCAACCCACGGGATTCCCTC	82	1741
455443	4772	4791	AAGCAACCCACGGGATTCCC	77	1742
455444	4774	4793	GTAAGCAACCCACGGGATTC	74	1743
455445	4776	4795	AGGTAAGCAACCCACGGGAT	76	1744
455446	4778	4797	GTAGGTAAGCAACCCACGGG	82	1745
455447	4780	4799	AGGTAGGTAAGCAACCCACG	88	1746
455448	4782	4801	ATAGGTAGGTAAGCAACCCA	83	1747
455449	4784	4803	TTATAGGTAGGTAAGCAACC	59	1748
455450	4786	4805	CCTTATAGGTAGGTAAGCAA	65	1749
455451	4788	4807	CACCTTATAGGTAGGTAAGC	62	1750
455452	4790	4809	ACCACCTTATAGGTAGGTAA	57	1751
455453	4792	4811	AAACCACCTTATAGGTAGGT	75	1752
455454	4794	4813	ATAAACCACCTTATAGGTAG	35	1753
455455	4796	4815	TTATAAACCACCTTATAGGT	39	1754
455456	4798	4817	GCTTATAAACCACCTTATAG	58	1755
455457	4800	4819	CAGCTTATAAACCACCTTAT	86	1756
455458	4802	4821	AGCAGCTTATAAACCACCTT	86	1757
455459	4804	4823	ACAGCAGCTTATAAACCACC	80	1758
455460	4806	4825	GGACAGCAGCTTATAAACCA	69	1759
455461	4808	4827	CAGGACAGCAGCTTATAAAC	72	1760
455462	4810	4829	GCCAGGACAGCAGCTTATAA	76	1761
455463	4812	4831	TGGCCAGGACAGCAGCTTAT	89	1762
455464	4814	4833	AGTGGCCAGGACAGCAGCTT	80	1763
455465	4816	4835	GCAGTGGCCAGGACAGCAGC	78	1764
455466	4818	4837	ATGCAGTGGCCAGGACAGCA	85	1765
455467	4820	4839	GAATGCAGTGGCCAGGACAG	80	1766
455468	4822	4841	TTGAATGCAGTGGCCAGGAC	83	1767
455469	4824	4843	ATTTGAATGCAGTGGCCAGG	84	1768
455470	4826	4845	GAATTTGAATGCAGTGGCCA	81	1769
455471	4828	4847	TGGAATTTGAATGCAGTGGC	85	1770
455472	4830	4849	ATTGGAATTTGAATGCAGTG	64	1771
455473	4832	4851	ACATTGGAATTTGAATGCAG	80	1772
455474	4834	4853	ACACATTGGAATTTGAATGC	73	1773
455475	4836	4855	GTACACATTGGAATTTGAAT	80	1774
455476	4838	4857	AAGTACACATTGGAATTTGA	77	1775
455477	4840	4859	TGAAGTACACATTGGAATTT	68	1776

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455478	4842	4861	TATGAAGTACACATTGGAAT	66	1777
455479	4844	4863	ACTATGAAGTACACATTGGA	83	1778
455480	4846	4865	ACACTATGAAGTACACATTG	76	1779
455481	4848	4867	TTACACTATGAAGTACACAT	78	1780
455482	4850	4869	TTTACACTATGAAGTACAC	76	1781
455483	4852	4871	ATTTTACACTATGAAGTAC	60	1782
455484	4854	4873	AAATTTTACACTATGAAGT	35	1783
455485	4856	4875	ATAAATTTTACACTATGAA	9	1784
455486	4858	4877	ATATAAATTTTACACTATG	0	1785
455487	4860	4879	TAATATAAATTTTACACTA	21	1786
455488	4862	4881	AATAATATAAATTTTACAC	10	1787
455489	4864	4883	ACAATAATATAAATTTTAC	7	1788
455490	4925	4944	AGTTAAAGTAGATACAGCAA	71	1789
455491	4927	4946	GAAGTTAAAGTAGATACAGC	63	1790
455492	4929	4948	TGGAAGTTAAAGTAGATACA	69	1791
455493	4931	4950	TCTGGAAGTTAAAGTAGATA	65	1792
455494	4933	4952	TTTCTGGAAGTTAAAGTAGA	55	1793
455495	4935	4954	TATTTCTGGAAGTTAAAGTA	57	1794
455496	4937	4956	TTATTTCTGGAAGTTAAAG	36	1795
455497	4939	4958	CGTTTATTTCTGGAAGTTAA	77	1796

Table 53

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 2

ISIS NO	Human Start Site	Human Stop Site	Sequence	% inhibition	SEQ ID NO
455498	917	936	CACGCCGTCATGCATAATTC	0	1797
455499	919	938	GGCACGCCGTCATGCATAAT	0	1798
455500	940	959	GCCCAGCCCCAGCCTGGCCG	35	1799
455501	962	981	ACAGCCCCTTCAGCCAATCC	15	1800
455502	964	983	TTACAGCCCCTTCAGCCAAT	14	1801
455503	966	985	AATTACAGCCCCTTCAGCCA	28	1802
455504	968	987	TGAATTACAGCCCCTTCAGC	6	1803
455505	970	989	GCTGAATTACAGCCCCTTCA	15	1804
455506	972	991	CCGCTGAATTACAGCCCCTT	4	1805
455507	974	993	AACCGCTGAATTACAGCCCC	8	1806
455508	976	995	GAAACCGCTGAATTACAGCC	16	1807
455509	978	997	CGGAAACCGCTGAATTACAG	24	1808
455510	980	999	TCCGGAAACCGCTGAATTAC	12	1809
455511	982	1001	GCTCCGGAAACCGCTGAATT	15	1810
455512	984	1003	CAGCTCCGGAAACCGCTGAA	23	1811
455513	986	1005	CGCAGCTCCGGAAACCGCTG	4	1812

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455514	988	1007	GCCGCAGCTCCGGAACCGC	13	1813
455515	1378	1397	AGTCCCTTCCGAGGCCCGCT	81	1814
455516	1408	1427	CGAAGAACGAAACTTCCTC	68	1815
455517	1697	1716	CAGACACACCTATTCCTGCC	82	1816
455518	1748	1767	TTATGCAATAAAGCCTACCC	70	1817
455519	1795	1814	TTAGAAAGAGTACCGGTCTG	75	1818
455520	1987	2006	AATGGCTCAATTATTTATCT	59	1819
455521	2083	2102	TTTACCCAAGATCTTGGCTC	76	1820
455522	2175	2194	ACTTCAGTGCAACCACACCC	70	1821
455523	2205	2224	CCAACCTGGGCGACGGTTTG	67	1822
455524	2281	2300	CTAACCACTGATTTTGTAC	56	1823
455525	2316	2335	GTACACACTATACACATTTT	85	1824
455526	2346	2365	CTTTAGTTGCACATACAGTA	80	1825
455527	2383	2402	GCCAAAAATTTACAACCCAT	86	1826
455528	2413	2432	TTCAAGCCCAATGCTTTATC	76	1827
455529	2561	2580	CTGGAACATGTAATAAGGAA	71	1828
455530	2669	2688	AGAGACTAAAATCAAGGCTC	87	1829
455531	2900	2919	TAGACTCTAGACCCAATTCC	77	1830
455532	3780	3799	GAAATGACCACTGATCAAGC	74	1831
455533	3867	3886	AAGTTGGTCACCACCTCTAC	81	1832
455534	4291	4310	AACTTATTCTTCATAGCAAC	58	1833
455535	4587	4606	TATTTGGGACCCAGTTGAAA	60	1834
455536	5000	5019	AGAACTGAAATTCCTTGGTC	88	1835
455537	5030	5049	AAGTTTTAAAAGCTTCCCCT	76	1836
455538	5554	5573	TCACCCAAAGTACCAAATCA	71	1837
455539	5667	5686	CAAAAGTTATGGTGAAATTT	44	1838
455540	5699	5718	AAGTACTCTTTCAGTGGTTT	88	1839
455541	6844	6863	AATTAAAGAGTTGCGGTAAT	68	1840
455542	6926	6945	GTTTCATGAAAACGGACAAT	78	1841
455543	7050	7069	AGGATTCAGTCCCAGATCTG	18	1842
455544	7282	7301	TCAATAATGATGACTTTCTC	72	1843
455545	7528	7547	TTAAACCCAATTATTAACAG	45	1844
455546	7624	7643	GTAAAACACACATTTTATAT	62	1845
455547	7682	7701	GTAAACAGAAAGGGCTGCAA	86	1846
455548	8078	8097	GGGCAGATTTACCTTCCTTA	89	1847
455549	8126	8145	GGGTAGCAGGAAGGAAAGCC	80	1848
455550	8214	8233	AATATAAGTTCTTTGGCTGA	60	1849
455551	8244	8263	TACAATAGCAATCACCTTAG	89	1850
455552	8284	8303	CCATGAAACCCTCAAACATA	75	1851
337332	66135	66154	GAAGCCCTTGCCAGCCATGT	91	1541
337333	66140	66159	AAGGAGAAGCCCTTGCCAGC	87	1542
345785	67129	67148	TGCCTCCTCCTTGGGAATGT	82	1543

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455246	74639	74658	CAAGGAGGCTGTAACTGAA	84	1545
455247	74641	74660	ACCAAGGAGGCTGTAACTG	78	1546
455248	74643	74662	GCACCAAGGAGGCTGTAAAC	69	1547
455249	74645	74664	AAGCACCAAGGAGGCTGTTA	83	1548
455250	74647	74666	TAAAGCACCAAGGAGGCTGT	77	1549
455251	74649	74668	CTTAAAGCACCAAGGAGGCT	78	1550
455252	74651	74670	TGCTTAAAGCACCAAGGAGG	80	1551
455253	74653	74672	AATGCTTAAAGCACCAAGGA	75	1552
455254	74655	74674	TGAATGCTTAAAGCACCAAG	80	1553
455255	74657	74676	GCTGAATGCTTAAAGCACCA	82	1554
455256	74659	74678	AAGCTGAATGCTTAAAGCAC	67	1555
455257	74661	74680	GGAAGCTGAATGCTTAAAGC	79	1556
455258	74663	74682	AAGGAAGCTGAATGCTTAAA	79	1557
455259	74665	74684	TGAAGGAAGCTGAATGCTTA	72	1558
455260	74667	74686	CCTGAAGGAAGCTGAATGCT	75	1559
455261	74714	74733	TAAGGGTTTGACCTGAAGCC	72	1560
455262	74764	74783	TAAACCTTCCTATTTCAACA	77	1561
455263	74766	74785	CTTAAACCTTCCTATTTCAA	64	1562
455264	74768	74787	TCCTTAAACCTTCCTATTTTC	73	1563
455265	74770	74789	TCTCCTTAAACCTTCCTATT	87	1564
455266	74772	74791	ATTCTCCTTAAACCTTCCTA	80	1565
455267	74774	74793	AGATTCTCCTTAAACCTTCC	87	1566
455268	74776	74795	TTAGATTCTCCTTAAACCTT	84	1567
455269	74778	74797	GCTTAGATTCTCCTTAAACC	87	1568
455270	74780	74799	ATGCTTAGATTCTCCTTAAA	87	1569
455271	74782	74801	AAATGCTTAGATTCTCCTTA	89	1570
455272	74784	74803	TAAATGCTTAGATTCTCCT	88	1571
455273	74826	74845	ATACATTACAAAGGAAAATA	12	1572
455274	74828	74847	CAATACATTACAAAGGAAAA	28	1573
455275	74860	74879	CACCCTCTGCCCAGCCTTAC	63	1574
455276	74862	74881	AGCACCTCTGCCCAGCCTT	79	1575
455277	74864	74883	TAAGCACCTCTGCCCAGCC	65	1576
455278	74866	74885	TGTAAGCACCTCTGCCCAG	62	1577
455279	74868	74887	GTTGTAAGCACCTCTGCCC	62	1578
455280	74870	74889	AGGTTGTAAGCACCTCTGC	75	1579
455281	74872	74891	CAAGGTTGTAAGCACCTCT	83	1580
455282	74874	74893	GTCAAGGTTGTAAGCACCT	86	1581
455283	74876	74895	GAGTCAAGGTTGTAAGCACC	69	1582
455284	74878	74897	GGGAGTCAAGGTTGTAAGCA	37	1583
455285	74880	74899	AAGGGAGTCAAGGTTGTAAG	56	1584
455286	74882	74901	GAAAGGGAGTCAAGGTTGTA	61	1585
455287	74884	74903	GAGAAAGGGAGTCAAGGTTG	56	1586

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455288	74896	74915	ATCAAGTCCAGGGAGAAAGG	55	1587
455289	74898	74917	AGATCAAGTCCAGGGAGAAA	69	1588
455290	74900	74919	GCAGATCAAGTCCAGGGAGA	80	1589
455291	74902	74921	CAGCAGATCAAGTCCAGGGA	90	1590
455292	74904	74923	AACAGCAGATCAAGTCCAGG	77	1591
455293	74906	74925	GAAACAGCAGATCAAGTCCA	81	1592
455294	74908	74927	CTGAAACAGCAGATCAAGTC	75	1593
455295	74910	74929	CTCTGAAACAGCAGATCAAG	76	1594
455296	74912	74931	GCCTCTGAAACAGCAGATCA	74	1595
455297	74914	74933	TAGCCTCTGAAACAGCAGAT	75	1596
455298	74916	74935	CCTAGCCTCTGAAACAGCAG	76	1597
455299	74918	74937	AACCTAGCCTCTGAAACAGC	83	1598
455300	74920	74939	ACAACCTAGCCTCTGAAACA	57	1599
455301	74922	74941	AAACAACCTAGCCTCTGAAA	72	1600
455302	74924	74943	AGAAACAACCTAGCCTCTGA	78	1601
455303	74926	74945	ACAGAAACAACCTAGCCTCT	69	1602
455304	74928	74947	CCACAGAAACAACCTAGCCT	70	1603
455305	74930	74949	ACCCACAGAAACAACCTAGC	80	1604
455306	74932	74951	GCACCCACAGAAACAACCTA	70	1605
455307	74934	74953	AGGCACCCACAGAAACAACC	75	1606
455308	74936	74955	TAAGGCACCCACAGAAACAA	70	1607
455309	74938	74957	GATAAGGCACCCACAGAAAC	65	1608
455310	74940	74959	CTGATAAGGCACCCACAGAA	66	1609
455311	74942	74961	CCCTGATAAGGCACCCACAG	81	1610
455312	74944	74963	AGCCCTGATAAGGCACCCAC	79	1611
455313	74946	74965	CCAGCCCTGATAAGGCACCC	74	1612
455314	74948	74967	TCCCAGCCCTGATAAGGCAC	74	1613
455315	74950	74969	TATCCAGCCCTGATAAGGC	66	1614
455316	74952	74971	AGTATCCCAGCCCTGATAAG	48	1615
455317	74954	74973	GAAGTATCCCAGCCCTGATA	63	1616
455318	74956	74975	CAGAAGTATCCCAGCCCTGA	82	1617
455319	74958	74977	ATCAGAAGTATCCCAGCCCT	80	1618
455320	75066	75085	GATTCCTAAAACAAACAGGA	37	1619
455321	75068	75087	AGGATTCCTAAAACAAACAG	42	1620
455322	75070	75089	CCAGGATTCCTAAAACAAAC	72	1621
455323	75072	75091	GACCAGGATTCCTAAAACAA	71	1622
455324	75074	75093	GAGACCAGGATTCCTAAAAC	43	1623
455325	75076	75095	CTGAGACCAGGATTCCTAAA	77	1624
455326	75078	75097	TCCTGAGACCAGGATTCCTA	76	1625
455327	75080	75099	GGTCCTGAGACCAGGATTCC	69	1626
455328	75082	75101	GAGGTCCTGAGACCAGGATT	76	1627
455329	75084	75103	ATGAGGTCCTGAGACCAGGA	81	1628

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455330	75086	75105	CCATGAGGTCCTGAGACCAG	84	1629
455331	75088	75107	TTCCATGAGGTCCTGAGACC	75	1630
455332	75090	75109	TCTTCCATGAGGTCCTGAGA	75	1631
455333	75092	75111	CTTCTTCCATGAGGTCCTGA	79	1632
455334	75094	75113	CTCTTCTTCCATGAGGTCCT	83	1633
455335	75096	75115	CCCTCTTCTTCCATGAGGTC	74	1634
455336	75098	75117	CCCCCTCTTCTTCCATGAGG	72	1635
455337	75100	75119	CTCCCCCTCTTCTTCCATGA	72	1636
455338	75164	75183	CCTGAGCTCAACCAGACACG	79	1637
455339	75166	75185	TCCCTGAGCTCAACCAGACA	73	1638
455340	75168	75187	ATCCCTGAGCTCAACCAGA	75	1639
455341	75170	75189	ATATTCCCTGAGCTCAACCA	65	1640
455342	75172	75191	CCATATTCCCTGAGCTCAAC	78	1641
455343	75174	75193	AACCATATTCCCTGAGCTCA	81	1642
455344	75176	75195	AGAACCATATTCCCTGAGCT	77	1643
455345	75178	75197	TAAGAACCATATTCCCTGAG	73	1644
455346	75180	75199	GCTAAGAACCATATTCCCTG	81	1645
455347	75254	75273	TCAGTAAGCCTTTGCCCTGC	79	1646
455348	75256	75275	TATCAGTAAGCCTTTGCCCT	72	1647
455349	75258	75277	TTTATCAGTAAGCCTTTGCC	76	1648
455350	75260	75279	AGTTTATCAGTAAGCCTTTG	84	1649
455351	75262	75281	CAAGTTTATCAGTAAGCCTT	82	1650
455352	75264	75283	CTCAAGTTTATCAGTAAGCC	82	1651
455353	75266	75285	GACTCAAGTTTATCAGTAAG	70	1652
455354	75268	75287	CAGACTCAAGTTTATCAGTA	78	1653
455355	75270	75289	GGCAGACTCAAGTTTATCAG	67	1654
455356	75272	75291	AGGGCAGACTCAAGTTTATC	51	1655
455357	75274	75293	CGAGGGCAGACTCAAGTTTA	54	1656
455358	75276	75295	TACGAGGGCAGACTCAAGTT	56	1657
455359	75278	75297	CATACGAGGGCAGACTCAAG	59	1658
455360	75280	75299	CTCATACGAGGGCAGACTCA	74	1659
455361	75282	75301	CCCTCATACGAGGGCAGACT	67	1660
455362	75309	75328	CAGCCTCAGAGGGAGGCCAG	40	1661
455363	75311	75330	ACCAGCCTCAGAGGGAGGCC	34	1662
455364	75313	75332	TCACCAGCCTCAGAGGGAGG	49	1663
455365	75315	75334	AGTCACCAGCCTCAGAGGGA	50	1664
455366	75412	75431	CCCATACGCACAGGAGAGGC	81	1665
455367	75414	75433	TTCCCATACGCACAGGAGAG	72	1666
455368	75416	75435	TGTTCCCATACGCACAGGAG	80	1667
455369	75418	75437	GGTGTTCCTACGCACAGG	76	1668
455370	75420	75439	TAGGTGTTCCTACGCACA	87	1669
455371	75422	75441	GCTAGGTGTTCCTACGCA	92	1670

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455372	75424	75443	GTGCTAGGTGTTCCCATACG	81	1671
455373	75491	75510	GAGGCAAGGTGGTTTTGAGT	55	1672
455374	75493	75512	CTGAGGCAAGGTGGTTTTGA	74	1673
455375	75495	75514	AGCTGAGGCAAGGTGGTTTT	79	1674
455376	75497	75516	TCAGCTGAGGCAAGGTGGTT	80	1675
455377	75499	75518	GATCAGCTGAGGCAAGGTGG	77	1676
455378	75501	75520	CTGATCAGCTGAGGCAAGGT	60	1677
455379	75503	75522	CTCTGATCAGCTGAGGCAAG	74	1678
455380	75505	75524	AACTCTGATCAGCTGAGGCA	77	1679
455381	75507	75526	GAAACTCTGATCAGCTGAGG	78	1680
455382	75509	75528	CAGAACTCTGATCAGCTGA	78	1681
455383	75547	75566	CAGAGACCAGCTAATTTGAT	69	1682
455384	75549	75568	TTCAGAGACCAGCTAATTTG	78	1683
455385	75551	75570	AATTCAGAGACCAGCTAATT	77	1684
455386	75553	75572	TTAATTCAGAGACCAGCTAA	83	1685
455387	75610	75629	CTCCAGGCAGGAGGACTGGG	79	1686
455388	75612	75631	GTCTCCAGGCAGGAGGACTG	65	1687
455389	75614	75633	CTGTCTCCAGGCAGGAGGAC	57	1688
455390	75616	75635	AACTGTCTCCAGGCAGGAGG	75	1689
455391	75618	75637	TCAACTGTCTCCAGGCAGGA	86	1690
455392	75620	75639	CATCAACTGTCTCCAGGCAG	80	1691
455393	75622	75641	CACATCAACTGTCTCCAGGC	86	1692
455394	75624	75643	GACACATCAACTGTCTCCAG	85	1693
455395	75658	75677	GAAGAGTGTTGCTGGAGAAG	73	1694
455396	75660	75679	CTGAAGAGTGTTGCTGGAGA	78	1695
455397	75662	75681	TACTGAAGAGTGTTGCTGGA	83	1696
455398	75664	75683	TGTACTGAAGAGTGTTGCTG	86	1697
455399	75666	75685	TATGTACTGAAGAGTGTTGC	74	1698
455400	75668	75687	ATTATGTACTGAAGAGTGTT	74	1699
455401	75670	75689	TTATTATGTACTGAAGAGTG	84	1700
455402	75672	75691	GCTTATTATGTACTGAAGAG	84	1701
455403	75674	75693	AAGCTTATTATGTACTGAAG	77	1702
455404	75676	75695	TTAAGCTTATTATGTACTGA	75	1703
455405	75678	75697	AGTTAAGCTTATTATGTACT	81	1704
455406	75680	75699	TCAGTTAAGCTTATTATGTA	58	1705
455407	75682	75701	TATCAGTTAAGCTTATTATG	65	1706
455408	75684	75703	TTTATCAGTTAAGCTTATTA	46	1707
455409	75686	75705	TGTTTATCAGTTAAGCTTAT	68	1708
455410	75688	75707	TCTGTTTATCAGTTAAGCTT	83	1709
455411	75726	75745	AACCCAATGGTAAGCCCAAG	87	1710
455412	75728	75747	TAAACCCAATGGTAAGCCCA	87	1711
455413	75730	75749	TTTAAACCCAATGGTAAGCC	78	1712

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455414	75732	75751	GATTTAAACCCAATGGTAAG	31	1713
455415	75734	75753	ATGATTTAAACCCAATGGTA	71	1714
455416	75736	75755	CTATGATTTAAACCCAATGG	67	1715
455417	75738	75757	CCCTATGATTTAAACCCAAT	70	1716
455418	75740	75759	GTCCCTATGATTTAAACCCA	83	1717
455419	75742	75761	AGGTCCCTATGATTTAAACC	64	1718
455420	75776	75795	TATCTGCTCCAGAGAAGCCC	76	1719
455421	75778	75797	AATATCTGCTCCAGAGAAGC	78	1720
455422	75801	75820	CTACCTAAGGCCATGAACTT	74	1721
455423	75803	75822	TGCTACCTAAGGCCATGAAC	82	1722
455424	75805	75824	CATGCTACCTAAGGCCATGA	84	1723
455425	75823	75842	CAGAGTTAAGACCAGATACA	84	1724
455426	75825	75844	ATCAGAGTTAAGACCAGATA	83	1725
455427	75827	75846	CAATCAGAGTTAAGACCAGA	77	1726
455428	75829	75848	TACAATCAGAGTTAAGACCA	81	1727
455429	75831	75850	GCTACAATCAGAGTTAAGAC	86	1728
455430	75833	75852	TTGCTACAATCAGAGTTAAG	85	1729
455431	75835	75854	TTTTGCTACAATCAGAGTTA	85	1730
455432	75837	75856	ACTTTTGCTACAATCAGAGT	73	1731
455433	75839	75858	GAACTTTGTCTACAATCAGA	80	1732
455434	75841	75860	CAGAACTTTTGCTACAATCA	82	1733
455435	75843	75862	CTCAGAACTTTTGCTACAAT	79	1734
455436	75845	75864	CTCTCAGAACTTTTGCTACA	76	1735
455437	75847	75866	TCCTCTCAGAACTTTTGCTA	75	1736
455438	75849	75868	GCTCCTCTCAGAACTTTTGC	85	1737
455439	75851	75870	CAGCTCCTCTCAGAACTTTT	85	1738
455440	75853	75872	CTCAGCTCCTCTCAGAACTT	80	1739
455441	75855	75874	GGCTCAGCTCCTCTCAGAAC	75	1740
455442	75957	75976	GCAACCCACGGGATTCCCTC	82	1741
455443	75959	75978	AAGCAACCCACGGGATTCCC	77	1742
455444	75961	75980	GTAAGCAACCCACGGGATTC	74	1743
455445	75963	75982	AGGTAAGCAACCCACGGGAT	76	1744
455446	75965	75984	GTAGGTAAGCAACCCACGGG	82	1745
455447	75967	75986	AGGTAGGTAAGCAACCCACG	88	1746
455448	75969	75988	ATAGGTAGGTAAGCAACCCA	83	1747
455449	75971	75990	TTATAGGTAGGTAAGCAACC	59	1748
455450	75973	75992	CCTTATAGGTAGGTAAGCAA	65	1749
455451	75975	75994	CACCTTATAGGTAGGTAAGC	62	1750
455452	75977	75996	ACCACCTTATAGGTAGGTAA	57	1751
455453	75979	75998	AAACCACCTTATAGGTAGGT	75	1752
455454	75981	76000	ATAAACACCTTATAGGTAG	35	1753
455455	75983	76002	TTATAAACACCTTATAGGT	39	1754

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455456	75985	76004	GCTTATAAACCACCTTATAG	58	1755
455457	75987	76006	CAGCTTATAAACCACCTTAT	86	1756
455458	75989	76008	AGCAGCTTATAAACCACCTT	86	1757
455459	75991	76010	ACAGCAGCTTATAAACCACC	80	1758
455460	75993	76012	GGACAGCAGCTTATAAACCA	69	1759
455461	75995	76014	CAGGACAGCAGCTTATAAAC	72	1760
455462	75997	76016	GCCAGGACAGCAGCTTATAA	76	1761
455463	75999	76018	TGGCCAGGACAGCAGCTTAT	89	1762
455464	76001	76020	AGTGGCCAGGACAGCAGCTT	80	1763
455465	76003	76022	GCAGTGGCCAGGACAGCAGC	78	1764
455466	76005	76024	ATGCAGTGGCCAGGACAGCA	85	1765
455467	76007	76026	GAATGCAGTGGCCAGGACAG	80	1766
455468	76009	76028	TTGAATGCAGTGGCCAGGAC	83	1767
455469	76011	76030	ATTTGAATGCAGTGGCCAGG	84	1768
455470	76013	76032	GAATTTGAATGCAGTGGCCA	81	1769
455471	76015	76034	TGGAATTTGAATGCAGTGGC	85	1770
455472	76017	76036	ATTGGAATTTGAATGCAGTG	64	1771
455473	76019	76038	ACATTGGAATTTGAATGCAG	80	1772
455474	76021	76040	ACACATTGGAATTTGAATGC	73	1773
455475	76023	76042	GTACACATTGGAATTTGAAT	80	1774
455476	76025	76044	AAGTACACATTGGAATTTGA	77	1775
455477	76027	76046	TGAAGTACACATTGGAATTT	68	1776
455478	76029	76048	TATGAAGTACACATTGGAAT	66	1777
455479	76031	76050	ACTATGAAGTACACATTGGA	83	1778
455480	76033	76052	ACACTATGAAGTACACATTG	76	1779
455481	76035	76054	TTACACTATGAAGTACACAT	78	1780
455482	76037	76056	TTTTACACTATGAAGTACAC	76	1781
455483	76039	76058	ATTTTACACTATGAAGTAC	60	1782
455484	76041	76060	AAATTTTACACTATGAAGT	35	1783
455485	76043	76062	ATAAATTTTACACTATGAA	9	1784
455486	76045	76064	ATATAAATTTTACACTATG	0	1785
455487	76047	76066	TAATATAAATTTTACACTA	21	1786
455488	76049	76068	AATAATATAAATTTTACAC	10	1787
455489	76051	76070	ACAATAATATAAATTTTAC	7	1788
455490	76112	76131	AGTTAAAGTAGATACAGCAA	71	1789
455491	76114	76133	GAAGTTAAAGTAGATACAGC	63	1790
455492	76116	76135	TGGAAGTTAAAGTAGATACA	69	1791
455493	76118	76137	TCTGGAAGTTAAAGTAGATA	65	1792
455494	76120	76139	TTTCTGGAAGTTAAAGTAGA	55	1793
455495	76122	76141	TATTTCTGGAAGTTAAAGTA	57	1794
455496	76124	76143	TTTATTTCTGGAAGTTAAAG	36	1795
455497	76126	76145	CGTTTATTTCTGGAAGTTAA	77	1796

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455553	9123	9142	ACCTGCCCCCTATGTATAAGC	89	1852
	11261	11280			
455554	9484	9503	TTTGTAATATCTAACAGATA	20	1853
455555	9630	9649	TATATGACAGCCTCAATTTC	68	1854
455556	9677	9696	GGCATTGTGTGTAACAGGAA	81	1855
455557	9746	9765	TGTTAAATATTACTTAAAAT	4	1856
455558	9776	9795	AATTCCTTGGGTGGTAATCC	81	1857
455559	10071	10090	GGAAAGTTACAGGACAGGAA	77	1858
455560	10352	10371	GAAATGGCTTCTACAAAAAC	47	1859
455561	10472	10491	GGTCAGAATACCACAACTA	80	1860
455562	10634	10653	AGTCTAATGCTTTTAGATTC	59	1861
455563	11567	11586	CATTGGAAACTTAGGGTAA	37	1862
455564	11597	11616	ATTCTCACTGGGTATAGAGG	72	1863
455565	11700	11719	TAGCATTAATCTTTCCTAGG	92	1864
455566	9886	9905	GACTCAAAATAAGGTCCTC	86	1865
	12369	12388			
455567	12430	12449	ACAGATTTATTCATATAAGC	62	1866
455568	14060	14079	AGATCCATAGATTCTTCTT	80	1867
455569	14129	14148	ATCTGAATCAGAATATCTGC	88	1868
455570	14190	14209	GAAGACTTTATATTCTATGG	59	1869
455571	14355	14374	TATCCTTAATATTCAGGTAC	82	1870
455572	14501	14520	TTATTAAGACATCTGAAATA	31	1871
455573	14701	14720	TTAAGTGACTACACATGGAT	76	1872
455574	14761	14780	GATAATGTAACAACCCTATC	42	1873
455575	14828	14847	CTGAAGCATGAATTCACATT	83	1874
455576	15316	15335	AAATTCCACTACTCATGAAA	62	1875
455577	15370	15389	CTTCAGAGAATATCTCATTT	83	1876
455578	15400	15419	CACATCATAGTTTTGCATGA	70	1877
455579	15525	15544	TCTGACCATAAAGTTTAAA	70	1878
455580	16568	16587	TTGGTTAATAATAATGTATC	44	1879
455581	16832	16851	TCACACATTTGTCAAAATCC	89	1880
455582	16863	16882	TATATAATTGTGTACTGGCA	93	1881
455583	16930	16949	TGCCAGTGGTTCAGCAGAGG	77	1882
455584	17215	17234	AATGTTTATAGCAGCTTTAT	56	1883
455585	17330	17349	GTCACCTTGAATATAGTTTG	79	1884
455586	17426	17445	GGCTAAAATCCAAAACACTG	65	1885
455587	18449	18468	AACAGTATTTGAGAAAACCTT	21	1886
455588	19883	19902	GGGCTACAACTCAATAACAA	63	1887
455589	20512	20531	AAGTCCTTATCATTTAGCTC	69	1888
455590	21035	21054	GATATTCCTCAAAGTGACAGG	75	1889
455591	21188	21207	ATAATGAGACTTTAGCACTC	86	1890
455592	21422	21441	AATCTAAACTTCCAGCCAGG	78	1891

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455593	21493	21512	ACAATAATGCATGCAAATGT	67	1892
455594	21675	21694	CACTGCTATTTCCCCAGCAA	89	1893
455595	21710	21729	CTTAAGCCCCATAAGAACAA	65	1894
455596	21823	21842	ATCTAAACAGCAACATCTC	57	1895
455597	23917	23936	TAGTGATTGAATGTAGACTT	81	1896
455598	23980	23999	TTAGGCCACTAAGTCTGAGC	83	1897
455599	24178	24197	CAGCTGAAATCAGCCTTTGA	69	1898
455600	24345	24364	AATCTAGCTAAGTCCATAAC	43	1899
455601	24504	24523	TGCTTGGATATATAGAAGTC	80	1900
455602	24578	24597	AGGTCACCTTTCCCTATACGA	81	1901
455603	24608	24627	AGAAGGAAGATTCTTTTCTC	73	1902
455604	24924	24943	CTAAGAGAGGCCAACTGAAAT	60	1903
455605	25063	25082	GGCTCGAGGGCCACTGAAGG	59	1904
455606	25093	25112	AGCAAGCACATTGTCATGTC	83	1905
455607	25132	25151	GGCTGCCAAACTTTTCAAAA	76	1906
455608	25626	25645	TTTGTCTTGCCTAAAATGC	45	1907
455609	25688	25707	TTCCTTCAAGTCAACTTATC	69	1908
455610	26031	26050	CCAGCCTACAGATGACTTTC	78	1909
455611	26061	26080	GCCAACTTTAGCCCCTTCCA	85	1910
455612	26104	26123	AATGCAAAATCTTTACCCTT	58	1911
455613	26139	26158	CCAGCTCAAAAACACACACT	80	1912
455614	26227	26246	GTTTGAAAAATTCAAGAATG	26	1913
455615	26388	26407	ATAGTGTCTGGCTCATAATA	48	1914
455616	26597	26616	TCAGGTCTCAAAAACACCA	84	1915
455617	26648	26667	TGGCTGGTACCAGCTGGTGG	76	1916
455618	26766	26785	ACAAATTCATCGAGCTAATG	52	1917
455619	26908	26927	AGAATAGCATGGATTGAAT	49	1918
455620	26999	27018	CACAACTTGATCTTGCCAC	77	1919
455626	36534	36553	GAATGTAAAGTATCTTGTTT	47	1920
455627	36578	36597	TATAAAATACACACTGGATT	57	1921
455628	36614	36633	GAAATGTGGCTGCTTCAAAC	36	1922
455629	36649	36668	TGGAGTCACTAGCCACATGT	71	1923
455630	36691	36710	GCATACAAATTTACTGAAAC	58	1924
455631	36904	36923	CAAGTTAAATCTGCCTCAC	62	1925
455632	36975	36994	GGCATGTATTGATTGCCCTC	68	1926
455633	37026	37045	AGTAAAAGCAGTGGCTGACG	60	1927
455634	37086	37105	CACCTGCCACAGGACAAATG	28	1928
455635	37755	37774	TTGCCCCAATTAGGCCAATA	76	1929
455636	37822	37841	AAGGGCTTAAATTCCACTGG	73	1930
455637	37873	37892	GTACTTTACATGTGCAGCAC	81	1931
455638	38268	38287	AATATATCCAAAATGTTATT	8	1932
455639	38694	38713	GCAGCATCCAACAGAAATAG	62	1933

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455640	39294	39313	GAGACTGAACACACGCAAAC	65	1934
455641	39324	39343	GTTCTCTGGGATAGTGAGAA	49	1935
455642	39792	39811	GAGAAACCCAGCCAGCTAAT	69	1936
455643	39937	39956	GGAAGATCTGCCTGAGATTC	46	1937
455644	40132	40151	TACAGCATCCAGCTCAGTGC	63	1938
455645	40633	40652	CCCAGTTTAGAACAATACAA	65	1939
455646	40866	40885	GTAGCCATTGCCCAACACAG	63	1940
455647	40901	40920	CACCACAAGTCCCAGTAGGG	58	1941
455648	40923	40942	TAAACCAAAGTGTGCATATG	11	1942
455649	41087	41106	AAGGACTTACCAATCTTGAC	7	1943
455650	41114	41133	ACCTAACAATTTGGAGAGTC	44	1944
455651	41239	41258	TTACAAGACCAAAGGGTGCC	68	1945
455652	41329	41348	AAATCAACCTTCAAGACATC	13	1946
455653	41397	41416	AAAAATATGTCTACCACATC	52	1947
455654	41431	41450	AAGTTCTAGCTATGACAGAA	23	1948
455655	41575	41594	AGCCTGCAGAACTATGAGCC	48	1949
455656	41629	41648	ATTGGAAGCTTGCTGAGGCC	44	1950
455657	41644	41663	CTGCCCTCCGCCATGATTGG	48	1951
455658	41747	41766	CGAGACAGTGAGTTCTTGTG	64	1952
455659	42067	42086	CTGGCCCTTCACCAAATCAG	62	1953
455660	42139	42158	GGTCAGATTTATTAGTACAA	65	1954
455661	42904	42923	ATCATACCTGAAGAACTGC	16	1955
455662	43059	43078	ATACAGAGCTTTGAGAAAGG	38	1956
455663	43194	43213	TGTAACAGTGAGAGTCATCT	71	1957
455664	43284	43303	TCTGAGTCTTTACACAGTAT	72	1958
455665	43724	43743	TTCATCAAGGAAAGCATTTA	31	1959
455666	43765	43784	TGGAGATGTGGACTGAACTG	19	1960
455667	43908	43927	CCTGGGCCGCGAGTGGCTGCA	63	1961
455668	43926	43945	GTTTTGTCTCAGGTCTCACC	75	1962
455669	43941	43960	CCAGACCAGGGATTGTGTTT	34	1963
455670	43974	43993	CTCATTATAAAGTTGTTTGA	55	1964
455671	44507	44526	TGTACTATGAAAGTTTGTCA	80	1965
455672	44525	44544	AATGATATTGGAATAATCTG	26	1966
455673	44540	44559	CTTTGGAAAAGTTTGAATGA	26	1967
455674	44583	44602	CAGCCTCATAAAATAAGCTG	19	1968
455675	45414	45433	TACTGAGAATAGTGTTTCAC	71	1969
455676	45440	45459	AAGACATCCTTATCTTTTGC	75	1970
455677	45512	45531	TTCCAATATTTGTACCCTCA	87	1971
455678	45626	45645	TACAATGGCCTTTCTAAACC	64	1972
455679	45712	45731	AGATCTTTACTTTTCATTACA	54	1973
455680	46058	46077	TATGCAAATTGCATACATTT	59	1974
455681	46091	46110	TTTCAGATATTTTCCATA	88	1975

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455682	46241	46260	GTGTATTTCAACCACAATTTT	78	1976
455683	46571	46590	TGTCTTTGAACATGATCTTC	67	1977
455684	46676	46695	GCATGACTAATTAACACATC	58	1978
455685	46759	46778	CAGAGCAAGTGGCAGGGCTG	69	1979
455686	46791	46810	CAGAGAGAGTAAAAATTGTT	49	1980
455687	46905	46924	CAGCAGAAAGCAGTTAAATT	56	1981
455688	46941	46960	CAGTAATGGTGAGGGTGATG	28	1982
455689	46956	46975	GGTCCCCATTTCTACAGTA	67	1983
455690	47307	47326	ACACCTGAGCATATCAGTTT	67	1984
455691	47400	47419	CAGAAAATCCTAGTGCTGCC	62	1985
455692	47424	47443	ATAAAATACAAAGGTTTTCC	23	1986
455693	47467	47486	TCCAAATTGACTTAAACCAC	74	1987
455694	47528	47547	TTGAAAACATCCTTGGGATA	44	1988
455695	47579	47598	CAGGCTGGATTTGGGCCACG	76	1989
455696	47649	47668	GCCACAGATAATGCATAAAT	39	1990
455697	47795	47814	CTGGGTTGAGGCCACAAATA	78	1991
455698	47929	47948	GTTTGTGTACTTATAATCCC	75	1992
455699	47974	47993	GACAAAATGACACACATCCT	72	1993
455700	48188	48207	TTTCACACAATTGATAACTT	57	1994
455701	48208	48227	CAGGCCAACACAGAAAGCTG	70	1995
455702	48277	48296	AGAAACCCACCTCTAATACC	31	1996
455703	48402	48421	GCCACACTTTCCATTCTAGT	90	1997
455704	48417	48436	TGGTTACCAGCTCAAGCCAC	72	1998
455705	48566	48585	CAGGTCTAGAGGCCTATCCC	73	1999
455706	48665	48684	TCTTCAAAGAACCCAGCACC	63	2000
455707	48697	48716	AGATGGAGAGAAAGACTCTG	61	2001
455708	48728	48747	CCCACAGTGACAGTGACTCA	89	2002
455709	48768	48787	CTTAGAAGTTTTGGGAAGGT	60	2003
455710	48802	48821	ATGGTCCCTATCCAAGCCCA	81	2004
455711	48828	48847	ATGGGCAACCATTCTCTTCC	80	2005
455712	49754	49773	GTTGGATGTCTACTTAAACG	63	2006
455713	49845	49864	GACCACATGTTCAAGTAAGA	68	2007
455714	49923	49942	AAACAGAGGCAGTGGTGCTG	62	2008
455715	50053	50072	CCAAAAAGGAGGTCAATGCA	30	2009
455716	50522	50541	GTATCCCCAAGAGAAGGCTC	59	2010
455717	50571	50590	TCAAATGAAGCCAAAACCTC	63	2011
455718	50774	50793	CACTTTCTAGAGATTTTAAC	1	2012
455719	51623	51642	TCAGATCTTGCATGTCTGCG	2	2013
455720	51753	51772	CCGCAAGTGAGCGAGACACA	49	2014
455721	51827	51846	CCACATTCTTTAGTCAACTC	59	2015
455722	51856	51875	CAGAAAACATTTCTCAGAC	3	2016
455723	52033	52052	ACCAGTTTTCTAGCCGATCT	90	2017

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455724	52056	52075	AGGAAAAGCTTCTTTCATCC	34	2018
455725	52071	52090	GCTTTCGAGAAAGAAAGGAA	44	2019
455726	53203	53222	TGGATGAAGGTAAAAGTGCA	42	2020
455727	53246	53265	TCACTATAGGGCCTTGCACA	53	2021
455728	53262	53281	AGCTGGTGCAACATGCTCAC	69	2022
455729	53329	53348	GCATTCTCATGTAGAGTTGC	0	2023
455730	53344	53363	GATATGAATAGACAGGCATT	63	2024
455731	53431	53450	ATTCCCAGAACTTAAGCTTC	40	2025
455732	53571	53590	ATTCCATCATTCTTTGATGG	47	2026
455733	53900	53919	TGCACAAGGAATAAGTGAAT	51	2027
455734	54378	54397	AGAAGGGCTTGAACATCATG	15	2028
455735	54577	54596	GAGCCCAGATATGCAGAACA	58	2029
455736	54592	54611	AAATGACAAGCATCTGAGCC	16	2030
455737	54632	54651	ATTTATACCACTAGGAGGCA	52	2031
455738	55241	55260	TTCAGTGACATTAAGAAAAG	28	2032
455739	55256	55275	ATCTTAAGTTTACAGTTCAG	64	2033
455740	55277	55296	GCATGAAATTTACAATTTTT	26	2034
455741	55418	55437	TCCTGCCAATAAATTAAGAA	0	2035
455742	55657	55676	GAAGTCAGCCCGCCTCTCAC	33	2036
455743	55841	55860	GTGTCCCTCAGTAAAATCTC	53	2037
455744	55877	55896	ATGACCCTGGCCACCAACTC	63	2038
455745	55961	55980	CAGAATCAGAGAGCAAGCAG	56	2039
455746	56125	56144	CCTTAAAATCCACAGGGAAG	5	2040
455747	56151	56170	TCCCCATCACTAAGCCTTAC	31	2041
455748	56203	56222	TAACACCTCACCTACAGGC	56	2042
455749	56287	56306	ACACCATACTAAGTTTCTGA	68	2043
455750	57995	58014	CTTGTCAATGCACACTTTAA	80	2044
455751	58074	58093	TCTAGTTCAAATGATGTCTG	66	2045
455752	58089	58108	AATAAAGACAGAGTCTCTAG	30	2046
455753	58106	58125	CAAAATGAAGATCTCTGAAT	23	2047
455754	58173	58192	AGCTTTGTGGCTTTGTTTCAG	60	2048
455755	58259	58278	TGAATGACATGTACAAGTAA	52	2049
455756	58377	58396	TGTGTAAGGACTATATACTC	64	2050
455757	58471	58490	TTCAGCACAGTAACATACTG	41	2051
455758	58496	58515	AGATGTGTTACAATTGCCTA	76	2052
455759	58696	58715	TTTACATCCTGAAAGGTATT	51	2053
455760	59471	59490	ATATGTACTTATTAAACCTA	18	2054
455761	59748	59767	ACAAAAGGAAGCCTCTAGGC	0	2055
455762	59913	59932	CCAAGTGTTTGAATTCTGCA	83	2056
455763	60155	60174	CAGGTTGATGTTTCTAATTC	60	2057
455764	60170	60189	CTACAGCTGAAAGAACAGGT	76	2058
455765	60249	60268	ATGTTCCAAGCCAGAGAGCT	54	2059

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455766	60323	60342	GGTGTGGAGAACAACCTCAGC	72	2060
455767	60373	60392	GGGAATTTGGAAAGCCCCAG	0	2061
455768	60392	60411	CAGCCGCAGGAGCTGGATGG	42	2062
455769	60407	60426	GGAGCCAAGCAGGGTCAGCC	73	2063
455770	60433	60452	GGAGAGAAAAACAGGGCACT	69	2064
455771	60448	60467	TATCCACCTCAGTGGGAGA	1	2065
455772	60602	60621	TCTGAATCAATGAAAAGCAG	79	2066
455773	60703	60722	CATCACAATTTTAAAAATG	0	2067
455774	61216	61235	GTATTTTAAAAACACATATA	0	2068
455775	61251	61270	CTTAATATACATATGAATAC	14	2069
455786	61340	61359	CAAATATCACAGAGACAGTC	88	2070
455787	61758	61777	GTACAGCAACCTTATTTTAA	5	2071
455788	61853	61872	TTAAATCCTGGGAATGGCAC	83	2072
455789	61959	61978	CTAATGTTGATGGGTATTTA	60	2073
455790	62043	62062	CATGGTTATGTGTATCTGCA	89	2074
455791	62067	62086	TTCACCTTGATGTGAAATGAA	18	2075
455792	62500	62519	TGCCAGGGACACAACCTTGCT	82	2076
455793	62595	62614	ATGGCATTTCAGTACTAACAG	59	2077
455794	62610	62629	TTTTCTCAGAGAGAATGGC	67	2078
455795	63284	63303	AGTCACAATCAGGGAAGCCT	77	2079
455796	63449	63468	AGTAATCATTCCACCTTCTC	70	2080
455797	63464	63483	CAGTGTTAAGCAAACAGTAA	41	2081
455798	63554	63573	ATACACACATCTTCTAAGCA	48	2082
455799	63576	63595	TCAAGTTTGCTGAAAGCTGA	48	2083
455800	63591	63610	ATAGAGATTTTCATATCAAG	41	2084
455801	64070	64089	ACAGGGAGGTCTCAGGAATC	77	2085
455802	64122	64141	TTTAAGACCTTGAGGCATT	36	2086
455803	64586	64605	AGGGATGGTGCTCATTGTCT	20	2087
455804	64810	64829	GCCGGATCCCTTTTCTGGGC	64	2088
455805	64955	64974	TGATCACCTCGACTGAAAAC	65	2089
455806	65058	65077	GTGCCACCTTCCAACACACA	74	2090
455807	65530	65549	CAGACAGGTGTATTTGGTGG	65	2091
455808	65895	65914	ACTTTGCAAAATTTAGCCCA	77	2092
455809	65928	65947	TCCCATTTCCACGAGAATTT	76	2093
455810	65972	65991	GCCTTCAAGCCAGAGCCCTC	76	2094
455811	65987	66006	GACCAAGAGTTCAGGGCCTT	59	2095
455812	66099	66118	GTAATGGGAAAGCCAAGTCT	51	2096
455813	66128	66147	TTGCCAGCCATGTTTCTCTG	67	2097
455814	66283	66302	AGGGCATCCATCCCCTGCCA	7	2098
455815	66664	66683	TCACTGGAGCAAGCAAAACA	64	2099
455816	66775	66794	GGTCATAGAAAATAAACTTG	62	2100
455817	66863	66882	AGTGTGAGACCCTGAACAC	53	2101

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455818	66918	66937	AGAGAAACTGCCCATTTTT	71	2102
455819	66948	66967	AGATCATGGAACCTACAGCT	18	2103
455820	66963	66982	GGACATGGGAAGGAAAGATC	27	2104
455821	67191	67210	CAACAACCTACCTGGGTCAGC	51	2105
455822	67271	67290	AGGCATTTGCCTATCTATCC	58	2106
455823	67334	67353	CCAACAAAAGCACTCACTAC	56	2107
455824	67773	67792	TGAAATCTGGGCCTCAAACC	78	2108
455825	67843	67862	GAAACCCTTTCTTCAGACCA	79	2109
455826	68621	68640	TCAAAACAGCAAGTGCTGAA	60	2110
455827	69053	69072	AACCCTAAAGGATCACATTA	43	2111
455828	69357	69376	CAAAGAGCCGTGTGGCAGGG	65	2112
455829	69395	69414	GACCAGCCGTGGGACCCCAA	84	2113
455830	69473	69492	CCACAGGAAGGGCGATGGTA	58	2114
455831	69498	69517	GCAGGAAAGGACCTGGCCTC	45	2115
455832	70567	70586	TTAGGGAGCTGACACCCTAG	56	2116
455833	70645	70664	CAATTCAGTGCAGAATTCAA	80	2117
455834	70675	70694	TCTGAGTTTACTTTGGGCCA	75	2118
455835	70725	70744	CATGATGACCATGTGAAAGA	82	2119
455836	70890	70909	CTGAATGCTTACACCAAGAG	83	2120
455837	70973	70992	CCAATTTTCTATGAGCTTTG	85	2121
455838	71013	71032	CTTTTATGTATAAAATAAGA	6	2122
455839	71573	71592	CCAGGTACATCTTCAATAGC	75	2123
455840	71610	71629	GTACAATTGCTTCAACTAGA	87	2124
455841	71698	71717	ACATTTTGGATGAGGGCAT	81	2125
455842	71750	71769	AAAGCCAAAGGTTATATCTC	77	2126
455843	71765	71784	AATGCTTGTGGTTCCAAAGC	79	2127
455844	71929	71948	TGTAAAAGTTTAAACAGCCTC	70	2128
455845	71992	72011	CATAACCTTTTCCCACCTGA	79	2129
455846	72036	72055	CAGTTCTTTGCACAAAGCTG	76	2130
455847	72127	72146	CAAGATTGTCTGGAAAGCTC	76	2131
455848	72202	72221	TCGCATTACAGTAAGCAGAGC	47	2132
455849	72229	72248	AAACCAGTTTCTTACTGAC	17	2133
455850	72285	72304	CGGTGTCACACAGATAAACT	73	2134
455851	72367	72386	TTAACTCTCACCCAGTGTCC	61	2135
455852	72406	72425	GTACTAAACATAGCCCAGGG	78	2136
455853	72687	72706	AAATACTCACCAAAGTCCCC	4	2137
455854	72768	72787	GTGACCAGCTCTCGGTGTGT	10	2138
455855	73340	73359	GATTTGGTTTGTCCAAACTG	49	2139
455856	73530	73549	GTCAGAAAAGCCAGATTTAC	46	2140
455857	73621	73640	GCAACTGGCAGGCCACGCCC	39	2141
455858	73636	73655	AGTTGTCCACCCTCTGCAAC	0	2142
455859	73683	73702	TGTCAAAGGTGAGGGACTCT	57	2143

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455860	74018	74037	ACACAAGACATTTTCCTTTT	64	1544
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Example 33: Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

Gapmers from the study described in Example 32 exhibiting significant *in vitro* inhibition of STAT3 were tested at various doses in HuVEC cells. Cells were plated at a density of 5,000 cells per well and transfected using LipofectAMINE2000® reagent with 1.1 nM, 3.3 nM, 10.0 nM, and 30.0 nM concentrations of antisense oligonucleotide, as specified in Table 54. After a treatment period of approximately 16 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199 (forward sequence ACATGCCACTTTGGTGTTCATAA, designated herein as SEQ ID NO: 6; reverse sequence TCTTCGTAGATTGTGCTGATAGAGAAC, designated herein as SEQ ID NO: 7; probe sequence CAGTATAGCCGCTTCCTGCAAGAGTCGAA, designated herein as SEQ ID NO: 8) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 54 and was calculated by plotting the concentrations of oligonucleotides used versus the percent inhibition of STAT3 mRNA expression achieved at each concentration, and noting the concentration of oligonucleotide at which 50% inhibition of STAT3 mRNA expression was achieved compared to the control. As illustrated in Table 54, STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 54
Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

ISIS No	1.1 nM	3.3 nM	10.0 nM	30.0 nM	IC ₅₀ (nM)
337332	7	19	46	80	10.4
345785	8	22	46	74	11.3
455265	20	43	64	85	5.0
455267	16	30	62	79	6.7
455269	23	49	72	84	4.0
455270	3	28	60	79	8.1
455271	16	40	71	86	4.9
455272	28	30	57	86	5.7
455282	18	28	55	80	7.4
455291	21	45	75	85	4.1
455370	6	23	53	78	9.0
455371	15	46	73	90	4.5
455391	10	30	54	75	8.5

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455393	6	33	62	81	7.0
455394	5	33	63	85	6.7
455398	7	25	56	76	8.8
455411	10	21	58	82	7.9
455412	15	27	50	79	8.4
455429	17	43	67	81	5.2
455438	20	43	66	83	5.0
455439	10	41	67	84	5.7
455447	7	23	53	87	7.7
455457	9	24	52	79	8.8
455458	8	34	62	83	6.7
455463	6	37	63	85	6.3
455471	11	42	67	78	5.9
455525	0	9	42	72	13.4
455527	0	21	60	87	7.8
455530	11	26	62	83	7.1
455536	5	21	62	85	7.6
455540	8	28	65	87	6.5
455547	6	19	45	67	13.4
455548	0	41	68	90	5.8
455551	0	3	33	72	15.9
455553	0	29	64	87	7.2
455565	0	19	54	86	8.8
455566	13	28	45	76	9.6
455569	0	16	47	76	11.1
455581	0	19	62	85	8.6
455582	0	26	70	89	6.9
455591	7	17	47	68	12.8
455594	0	16	48	76	10.9
455611	14	43	68	81	5.4
455637	10	22	56	76	8.9
455677	0	18	46	72	11.9
455681	16	19	42	69	13.0
455703	9	40	72	92	5.1
455708	11	15	45	77	10.7
455723	3	9	33	68	17.0
455762	0	9	42	70	14.1
455786	21	32	50	79	7.4
455790	13	19	56	84	7.8
455840	17	30	52	77	7.9

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Example 34: Antisense inhibition of human STAT3 in HuVEC cells by oligonucleotides designed by microwalk

Additional gapmers were designed based on the gapmers presented in Example 1 that demonstrated an inhibition of at least 50%. These gapmers were designed by creating gapmers shifted slightly upstream and downstream (i.e., “microwalk”) of the original gapmers. These gapmers were tested *in vitro*. ISIS 337332 was also included in the assay as a comparator. Cultured HuVEC cells at a density of 5,000 cells per well were transfected using LipofectAMINE 2000® reagent with 30 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. The human primer probe set RTS199, described hereinabove, was used to measure STAT3 mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells. The results are presented in Table 55.

The chimeric antisense oligonucleotides in Table 55 were designed as 5-10-5 MOE gapmers. The gapmers designated with an asterisk (*) in Table 55 are the original gapmers from which gapmers, ISIS 465226-466744, were designed via microwalk. The 5-10-5 gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5'-methylcytosines. “Target start site” indicates the 5'-most nucleoside to which the gapmer is targeted. “Target stop site” indicates the 3'-most nucleoside to which the gapmer is targeted. Each gapmer listed in Table 55 is targeted to the target region spanning nucleobases 2313-76017 of SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_010755.14 truncated from nucleotides 4185000 to 4264000).

Table 55

Inhibition of human STAT3 mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 2

ISIS No	Start Site	Stop Site	Sequence	% inhibition	SEQ ID NO
466646	2313	2332	CACACTATACACATTTTAA	3	2144
466647	2314	2333	ACACACTATACACATTTTA	11	2145
466648	2315	2334	TACACACTATACACATTTT	8	2146
455525*	2316	2335	GTACACACTATACACATTT	47	1824
466649	2317	2336	GGTACACACTATACACATT	46	2147
466650	2318	2337	AGGTACACACTATACACATT	46	2148
466651	2319	2338	CAGGTACACACTATACACAT	54	2149
466652	2320	2339	GCAGGTACACACTATACACA	68	2150
466653	2321	2340	AGCAGGTACACACTATACAC	43	2151
466654	2322	2341	CAGCAGGTACACACTATACA	56	2152

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466655	2323	2342	CCAGCAGGTACACACTATAC	72	2153
466656	2324	2343	ACCAGCAGGTACACACTATA	52	2154
466657	2325	2344	GACCAGCAGGTACACACTAT	69	2155
466658	2326	2345	AGACCAGCAGGTACACACTA	15	2156
466659	2327	2346	AAGACCAGCAGGTACACACT	49	2157
466660	2328	2347	TAAGACCAGCAGGTACACAC	59	2158
466661	2329	2348	GTAAGACCAGCAGGTACACA	73	2159
466662	2330	2349	AGTAAGACCAGCAGGTACAC	65	2160
466663	2331	2350	CAGTAAGACCAGCAGGTACA	64	2161
466664	2332	2351	ACAGTAAGACCAGCAGGTAC	53	2162
466665	2333	2352	TACAGTAAGACCAGCAGGTA	67	2163
466666	2334	2353	ATACAGTAAGACCAGCAGGT	75	2164
466667	2335	2354	CATACAGTAAGACCAGCAGG	66	2165
466668	2336	2355	ACATACAGTAAGACCAGCAG	55	2166
466669	2337	2356	CACATACAGTAAGACCAGCA	71	2167
466670	2338	2357	GCACATACAGTAAGACCAGC	83	2168
466671	2339	2358	TGCACATACAGTAAGACCAG	28	2169
466672	2340	2359	TTGCACATACAGTAAGACCA	70	2170
466673	2341	2360	GTTGCACATACAGTAAGACC	39	2171
466674	2342	2361	AGTTGCACATACAGTAAGAC	53	2172
466675	2343	2362	TAGTTGCACATACAGTAAGA	43	2173
455527*	2383	2402	GCCAAAAATTTACAACCCAT	48	1826
465806	2384	2403	AGCCAAAAATTTACAACCCA	29	2174
465807	2385	2404	CAGCCAAAAATTTACAACCC	7	2175
465808	2386	2405	CCAGCCAAAAATTTACAACC	35	2176
465809	2387	2406	GCCAGCCAAAAATTTACAAC	10	2177
465810	2388	2407	AGCCAGCCAAAAATTTACAA	37	2178
465811	2389	2408	CAGCCAGCCAAAAATTTACA	29	2179
465812	2390	2409	ACAGCCAGCCAAAAATTTAC	3	2180
465813	2391	2410	CACAGCCAGCCAAAAATTTA	6	2181
465814	2392	2411	GCACAGCCAGCCAAAAATTT	35	2182
465815	2393	2412	AGCACAGCCAGCCAAAAATT	22	2183
465816	2394	2413	CAGCACAGCCAGCCAAAAAT	23	2184
465817	2395	2414	TCAGCACAGCCAGCCAAAAA	33	2185
465818	2396	2415	ATCAGCACAGCCAGCCAAAA	32	2186
465819	2397	2416	TATCAGCACAGCCAGCCAAA	48	2187
465820	2398	2417	TTATCAGCACAGCCAGCCAA	32	2188
465821	2399	2418	TTTATCAGCACAGCCAGCCA	0	2189
465822	2400	2419	CTTTATCAGCACAGCCAGCC	49	2190
465823	2401	2420	GCTTTATCAGCACAGCCAGC	69	2191
465824	2402	2421	TGCTTTATCAGCACAGCCAG	48	2192
465825	2403	2422	ATGCTTTATCAGCACAGCCA	74	2193

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465826	2404	2423	AATGCTTTATCAGCACAGCC	62	2194
465827	2405	2424	CAATGCTTTATCAGCACAGC	67	2195
465828	2406	2425	CCAATGCTTTATCAGCACAG	71	2196
465829	2407	2426	CCCAATGCTTTATCAGCACA	47	2197
465830	2408	2427	GCCCAATGCTTTATCAGCAC	81	2198
465831	2409	2428	AGCCCAATGCTTTATCAGCA	75	2199
465832	2410	2429	AAGCCCAATGCTTTATCAGC	57	2200
465349	2655	2674	AGGCTCCAACCTCTAAAACA	41	2201
465350	2656	2675	AAGGCTCCAACCTCTAAAAC	34	2202
465351	2657	2676	CAAGGCTCCAACCTCTAAAA	43	2203
465352	2658	2677	TCAAGGCTCCAACCTCTAAA	51	2204
465353	2659	2678	ATCAAGGCTCCAACCTCTAA	38	2205
465354	2660	2679	AATCAAGGCTCCAACCTCTA	29	2206
465355	2661	2680	AAATCAAGGCTCCAACCTCT	56	2207
465356	2662	2681	AAAATCAAGGCTCCAACCTC	24	2208
465357	2663	2682	TAAAATCAAGGCTCCAACCT	46	2209
465358	2664	2683	CTAAAATCAAGGCTCCAACC	45	2210
465359	2665	2684	ACTAAAATCAAGGCTCCAAC	50	2211
465366	2666	2685	GACTAAAATCAAGGCTCCAA	51	2212
465367	2667	2686	AGACTAAAATCAAGGCTCCA	64	2213
465368	2668	2687	GAGACTAAAATCAAGGCTCC	76	2214
455530*	2669	2688	AGAGACTAAAATCAAGGCTC	74	1829
455536*	5000	5019	AGAAGTGAATTCCTTGGTC	52	1835
465833	5001	5020	CAGAACTGAAATTCCTTGGT	81	2215
465834	5002	5021	ACAGAACTGAAATTCCTTGG	81	2216
465835	5003	5022	AACAGAACTGAAATTCCTTG	48	2217
465836	5004	5023	GAACAGAACTGAAATTCCTT	46	2218
465837	5005	5024	AGAACAGAACTGAAATTCCT	39	2219
465838	5006	5025	AAGAACAGAACTGAAATTC	22	2220
465839	5007	5026	AAAGAACAGAACTGAAATTC	3	2221
465840	5008	5027	AAAAGAACAGAACTGAAATT	0	2222
465841	5009	5028	CAAAAGAACAGAACTGAAAT	0	2223
465842	5010	5029	ACAAAAGAACAGAACTGAAA	0	2224
465843	5011	5030	TACAAAAGAACAGAACTGAA	3	2225
465844	5012	5031	CTACAAAAGAACAGAACTGA	0	2226
465845	5013	5032	CCTACAAAAGAACAGAACTG	13	2227
465846	5014	5033	CCCTACAAAAGAACAGAACT	0	2228
465847	5015	5034	CCCCTACAAAAGAACAGAAC	7	2229
465848	5016	5035	TCCCCTACAAAAGAACAGAA	33	2230
465849	5017	5036	TTCCCCTACAAAAGAACAGA	18	2231
465850	5018	5037	CTTCCCCTACAAAAGAACAG	0	2232
465851	5019	5038	GCTTCCCCTACAAAAGAACA	43	2233

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465852	5020	5039	AGCTTCCCCTACAAAAGAAC	32	2234
465853	5021	5040	AAGCTTCCCCTACAAAAGAA	0	2235
465854	5022	5041	AAAGCTTCCCCTACAAAAGA	15	2236
465855	5023	5042	AAAAGCTTCCCCTACAAAAG	14	2237
465856	5024	5043	TAAAAGCTTCCCCTACAAAA	4	2238
465857	5025	5044	TTAAAAGCTTCCCCTACAAA	0	2239
465858	5026	5045	TTTAAAAGCTTCCCCTACAA	11	2240
465859	5027	5046	TTTTAAAAGCTTCCCCTACA	11	2241
465860	5688	5707	CAGTGGTTTTTATAAATGAC	29	2242
465861	5689	5708	TCAGTGGTTTTTATAAATGA	19	2243
465862	5690	5709	TTCAGTGGTTTTTATAAATG	4	2244
465863	5691	5710	TTTCAGTGGTTTTTATAAAT	0	2245
465864	5692	5711	CTTTCAGTGGTTTTTATAAA	0	2246
465865	5693	5712	TCTTTCAGTGGTTTTTATAA	0	2247
465866	5694	5713	CTCTTTCAGTGGTTTTTATA	35	2248
465867	5695	5714	ACTCTTTCAGTGGTTTTTAT	67	2249
465868	5696	5715	TACTCTTTCAGTGGTTTTTA	60	2250
465886	5697	5716	GTA CTCTTTCAGTGGTTTTT	85	2251
465887	5698	5717	AGTACTCTTTCAGTGGTTTT	62	2252
455540*	5699	5718	AAGTACTCTTTCAGTGGTTT	76	1839
465888	5700	5719	CAAGTACTCTTTCAGTGGTT	80	2253
465906	5701	5720	TCAAGTACTCTTTCAGTGGT	74	2254
465926	5702	5721	CTCAAGTACTCTTTCAGTGG	80	2255
465927	5703	5722	CCTCAAGTACTCTTTCAGTG	71	2256
465928	5704	5723	CCCTCAAGTACTCTTTCAGT	54	2257
465929	5705	5724	TCCCTCAAGTACTCTTTCAG	33	2258
465930	5706	5725	GTCCCTCAAGTACTCTTTCA	56	2259
465931	5707	5726	TGTCCCTCAAGTACTCTTTC	43	2260
465932	5708	5727	ATGTCCCTCAAGTACTCTTT	33	2261
465486	7674	7693	AAAGGGCTGCAAAAAATCTG	39	2262
465487	7675	7694	GAAAGGGCTGCAAAAAATCT	11	2263
465488	7676	7695	AGAAAGGGCTGCAAAAAATC	28	2264
465489	7677	7696	CAGAAAGGGCTGCAAAAAAT	39	2265
465490	7678	7697	ACAGAAAGGGCTGCAAAAAA	29	2266
465506	7679	7698	AACAGAAAGGGCTGCAAAAA	36	2267
465507	7680	7699	AAACAGAAAGGGCTGCAAAA	35	2268
465508	7681	7700	TAAACAGAAAGGGCTGCAAA	47	2269
455547*	7682	7701	GTAAACAGAAAGGGCTGCAA	72	1846
465509	7683	7702	GGTAAACAGAAAGGGCTGCA	70	2270
465510	7684	7703	TGGTAAACAGAAAGGGCTGC	63	2271
465511	7685	7704	CTGGTAAACAGAAAGGGCTG	60	2272
465526	7686	7705	CCTGGTAAACAGAAAGGGCT	65	2273

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465527	7687	7706	ACCTGGTAAACAGAAAGGGC	26	2274
465528	7688	7707	AACCTGGTAAACAGAAAGGG	53	2275
465529	7689	7708	TAACCTGGTAAACAGAAAGG	35	2276
465530	7690	7709	ATAACCTGGTAAACAGAAAG	3	2277
465531	7691	7710	GATAACCTGGTAAACAGAAA	17	2278
465532	7692	7711	AGATAACCTGGTAAACAGAA	14	2279
465533	7693	7712	AAGATAACCTGGTAAACAGA	26	2280
455548*	8078	8097	GGGCAGATTTACCTTCCTTA	77	1847
466722	8241	8260	AATAGCAATCACCTTAGGAA	53	2281
466723	8242	8261	CAATAGCAATCACCTTAGGA	62	2282
466724	8243	8262	ACAATAGCAATCACCTTAGG	48	2283
455551*	8244	8263	TACAATAGCAATCACCTTAG	65	1850
466725	8245	8264	CTACAATAGCAATCACCTTA	15	2284
466726	8246	8265	ACTACAATAGCAATCACCTT	45	2285
466727	8247	8266	AACTACAATAGCAATCACCT	42	2286
466728	8248	8267	AAACTACAATAGCAATCACC	26	2287
466729	8249	8268	AAAACCTACAATAGCAATCAC	14	2288
466730	8250	8269	CAAAACTACAATAGCAATCA	0	2289
466731	8251	8270	TCAAAACTACAATAGCAATC	29	2290
466732	8252	8271	TTCAAAACTACAATAGCAAT	20	2291
466733	8253	8272	TTTCAAAACTACAATAGCAA	14	2292
466734	8254	8273	GTTTCAAAACTACAATAGCA	58	2293
466735	8255	8274	TGTTTCAAAACTACAATAGC	28	2294
466736	8256	8275	GTGTTTCAAAACTACAATAG	42	2295
466737	8257	8276	AGTGTTTCAAAACTACAATA	13	2296
466738	8258	8277	AAGTGTTTCAAAACTACAAT	18	2297
466739	8259	8278	CAAGTGTTTCAAAACTACAA	30	2298
466740	8260	8279	CCAAGTGTTTCAAAACTACA	49	2299
466741	8261	8280	ACCAAGTGTTTCAAAACTAC	46	2300
466742	8262	8281	AACCAAGTGTTTCAAAACTA	41	2301
466743	8263	8282	CAACCAAGTGTTTCAAAACT	13	2302
455553*	9123	9142	ACCTGCCCCTATGTATAAGC	75	1852
	11261	11280			
466744	9124	9143	CACCTGCCCCTATGTATAAG	67	2303
	11262	11281			
466745	9125	9144	CCACCTGCCCCTATGTATAA	69	2304
	11263	11282			
466746	9126	9145	TCCACCTGCCCCTATGTATA	68	2305
	11264	11283			
466747	9127	9146	TTCCACCTGCCCCTATGTAT	69	2306
	11265	11284			
466748	9128	9147	ATTCCACCTGCCCCTATGTA	58	2307

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	11266	11285			
466749	9129	9148	TATTCCACCTGCCCCTATGT	38	2308
	11267	11286			
466750	9130	9149	TTATTCCACCTGCCCCTATG	47	309
	11268	11287			
466751	9131	9150	TTTATTCCACCTGCCCCTAT	54	2310
466752	9132	9151	TTTTATTCCACCTGCCCCTA	50	2311
466753	9133	9152	GTTTTATTCCACCTGCCCCT	58	2312
466754	9134	9153	TGTTTTATTCCACCTGCCCC	53	2313
466755	9135	9154	ATGTTTTATTCCACCTGCCC	69	2314
466756	9136	9155	TATGTTTTATTCCACCTGCC	3	2315
466757	9137	9156	TTATGTTTTATTCCACCTGC	48	2316
466758	9138	9157	ATTATGTTTTATTCCACCTG	53	2317
466759	9139	9158	AATTATGTTTTATTCCACCT	24	2318
466760	9140	9159	TAATTATGTTTTATTCCACC	10	2319
466761	9141	9160	CTAATTATGTTTTATTCCAC	13	2320
466762	9142	9161	CCTAATTATGTTTTATTCCA	23	2321
466763	9143	9162	TCCTAATTATGTTTTATTCC	27	2322
466764	9144	9163	CTCCTAATTATGTTTTATTCC	21	2323
466765	9145	9164	CCTCCTAATTATGTTTTATT	30	2324
465740	9862	9881	TGGCTTCTTCCTGAGACACA	81	2325
	12345	12364			
465741	9863	9882	TTGGCTTCTTCCTGAGACAC	68	2326
	12346	12365			
465742	9864	9883	GTTGGCTTCTTCCTGAGACA	81	2327
	12347	12366			
465743	9865	9884	TGTTGGCTTCTTCCTGAGAC	68	2328
	12348	12367			
465744	9866	9885	CTGTTGGCTTCTTCCTGAGA	44	2329
	12349	12368			
465745	9867	9886	CCTGTTGGCTTCTTCCTGAG	73	2330
	12350	12369			
465746	9868	9887	TCCTGTTGGCTTCTTCCTGA	61	2331
	12351	12370			
465747	9869	9888	CTCCTGTTGGCTTCTTCCTG	53	2332
	12352	12371			
465748	9870	9889	CCTCCTGTTGGCTTCTTCCT	78	2333
	12353	12372			
465749	9871	9890	TCCTCCTGTTGGCTTCTTCC	73	2334
	12354	12373			
465750	9872	9891	TTCCTCCTGTTGGCTTCTTC	70	2335
	12355	12374			

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465751	9873	9892	G TTCCTCCTGTTGGCTTCTT	89	2336
	12356	12375			
465752	9874	9893	GGTTCCTCCTGTTGGCTTCT	86	2337
	12357	12376			
465753	9875	9894	AGGTCCTCCTGTTGGCTTC	73	2338
	12358	12377			
465754	9876	9895	AAGGTCCTCCTGTTGGCTT	85	2339
	12359	12378			
465755	9877	9896	TAAGGTCCTCCTGTTGGCT	82	2340
	12360	12379			
465756	9878	9897	ATAAGGTCCTCCTGTTGGC	72	2341
	12361	12380			
465757	9879	9898	AATAAGGTCCTCCTGTTGG	61	2342
	12362	12381			
465758	9880	9899	AAATAAGGTCCTCCTGTTG	40	2343
	12363	12382			
465759	9881	9900	AAAATAAGGTCCTCCTGTT	41	2344
	12364	12383			
465760	9882	9901	CAAAATAAGGTCCTCCTGT	20	2345
	12365	12384			
465761	9883	9902	TCAAAATAAGGTCCTCCTG	57	2346
	12366	12385			
465762	9884	9903	CTCAAAATAAGGTCCTCCT	48	2347
	12367	12386			
465763	9885	9904	ACTCAAAATAAGGTCCTCC	52	2348
	12368	12387			
455566*	9886	9905	GACTCAAAATAAGGTCCTC	59	1855
	12369	12388			
465764	9887	9906	TGACTCAAAATAAGGTCCT	54	2349
	12370	12389			
465765	9888	9907	CTGACTCAAAATAAGGTTCC	47	2350
	12371	12390			
465766	9889	9908	CCTGACTCAAAATAAGG TTC	55	2351
	12372	12391			
465767	9890	9909	ACCTGACTCAAAATAAGGTT	48	2352
	12373	12382			
455553*	9123	9142	ACCTGCCCCTATGTATAAGC	75	1852
	11261	11280			
466744	9124	9143	CACCTGCCCCTATGTATAAG	67	2303
	11262	11281			
466745	9125	9144	CCACCTGCCCCTATGTATAA	69	2304
	11263	11282			

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466746	9126	9145	TCCACCTGCCCCTATGTATA	68	2305
	11264	11283			
466747	9127	9146	TTCCACCTGCCCCTATGTAT	69	2306
	11265	11284			
466748	9128	9147	ATTCCACCTGCCCCTATGTA	58	2307
	11266	11285			
466749	9129	9148	TATTCCACCTGCCCCTATGT	38	2308
	11267	11286			
466750	9130	9149	TTATTCCACCTGCCCCTATG	47	2309
	11268	11287			
465726	11695	11714	TTAATCTTTCCTAGGCAAAG	19	2353
465727	11696	11715	ATTAATCTTTCCTAGGCAAA	22	2354
465728	11697	11716	CATTAATCTTTCCTAGGCAA	43	2355
465729	11698	11717	GCATTAATCTTTCCTAGGCA	68	2356
465730	11699	11718	AGCATTAATCTTTCCTAGGC	80	2357
455565*	11700	11719	TAGCATTAATCTTTCCTAGG	74	1864
465731	11701	11720	TTAGCATTAATCTTTCCTAG	42	2358
465732	11702	11721	ATTAGCATTAATCTTTCCTA	22	2359
465733	11703	11722	GATTAGCATTAATCTTTCCT	40	2360
465734	11704	11723	AGATTAGCATTAATCTTTC	0	2361
465735	11705	11724	AAGATTAGCATTAATCTTTC	10	2362
465736	11706	11725	TAAGATTAGCATTAATCTTT	3	2363
465737	12342	12361	CTTCTTCCTGAGACACAGCC	71	2364
465738	12343	12362	GCTTCTTCCTGAGACACAGC	74	2365
465739	12344	12363	GGCTTCTTCCTGAGACACAG	83	2366
465740	9862	9881	TGGCTTCTTCCTGAGACACA	81	2325
	12345	12364			
465741	9863	9882	TTGGCTTCTTCCTGAGACAC	68	2326
	12346	12365			
465742	9864	9883	GTTGGCTTCTTCCTGAGACA	81	2327
	12347	12366			
465743	9865	9884	TGTTGGCTTCTTCCTGAGAC	68	2328
	12348	12367			
465744	9866	9885	CTGTTGGCTTCTTCCTGAGA	44	2329
	12349	12368			
465745	9867	9886	CCTGTTGGCTTCTTCCTGAG	73	2330
	12350	12369			
465746	9868	9887	TCCTGTTGGCTTCTTCCTGA	61	2331
	12351	12370			
465747	9869	9888	CTCCTGTTGGCTTCTTCCTG	53	2332
	12352	12371			
465748	9870	9889	CCTCCTGTTGGCTTCTTCCT	78	2333

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	12353	12372			
465749	9871	9890	TCCTCCTGTTGGCTTCTTCC	73	2334
	12354	12373			
465750	9872	9891	TTCCTCCTGTTGGCTTCTTC	70	2335
	12355	12374			
465751	9873	9892	GTTCTCCTGTTGGCTTCTT	89	2336
	12356	12375			
465752	9874	9893	GGTTCCTCCTGTTGGCTTCT	86	2337
	12357	12376			
465753	9875	9894	AGGTCCTCCTGTTGGCTTC	73	2338
	12358	12377			
465754	9876	9895	AAGGTCCTCCTGTTGGCTT	85	2339
	12359	12378			
465755	9877	9896	TAAGGTCCTCCTGTTGGCT	82	2340
	12360	12379			
465756	9878	9897	ATAAGGTCCTCCTGTTGGC	72	2341
	12361	12380			
465757	9879	9898	AATAAGGTCCTCCTGTTGG	61	2342
	12362	12381			
465758	9880	9899	AAATAAGGTCCTCCTGTTG	40	2343
	12363	12382			
465759	9881	9900	AAAATAAGGTCCTCCTGTT	41	2344
	12364	12383			
465760	9882	9901	CAAATAAGGTCCTCCTGT	20	2345
	12365	12384			
465761	9883	9902	TCAAATAAGGTCCTCCTG	57	2346
	12366	12385			
465762	9884	9903	CTCAAATAAGGTCCTCCT	48	2347
	12367	12386			
465763	9885	9904	ACTCAAATAAGGTCCTCC	52	2348
	12368	12387			
455566*	9886	9905	GACTCAAATAAGGTCCTC	59	1865
	12369	12388			
465764	9887	9906	TGACTCAAATAAGGTCCT	54	2349
	12370	12389			
465765	9888	9907	CTGACTCAAATAAGGTTCC	47	2350
	12371	12390			
465766	9889	9908	CCTGACTCAAATAAGG TTC	55	2351
	12372	12391			
465767	9890	9909	ACCTGACTCAAATAAGGTT	48	2352
	12373	12392			
465369	14101	14120	TGAGGATGACCCAGATAAA	64	2367

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465370	14102	14121	GTGAGGATGACCCCAGATAA	60	2368
465371	14103	14122	TGTGAGGATGACCCCAGATA	47	2369
465372	14104	14123	CTGTGAGGATGACCCCAGAT	68	2370
465373	14105	14124	CCTGTGAGGATGACCCCAGA	67	2371
465374	14106	14125	GCCTGTGAGGATGACCCCAG	70	2372
465375	14107	14126	TGCCTGTGAGGATGACCCCA	75	2373
465376	14108	14127	ATGCCTGTGAGGATGACCCC	72	2374
465377	14109	14128	TATGCCTGTGAGGATGACCC	58	2375
465378	14110	14129	CTATGCCTGTGAGGATGACC	56	2376
465379	14111	14130	GCTATGCCTGTGAGGATGAC	65	2377
465380	14112	14131	TGCTATGCCTGTGAGGATGA	23	2378
465386	14113	14132	CTGCTATGCCTGTGAGGATG	64	2379
465387	14114	14133	TCTGCTATGCCTGTGAGGAT	66	2380
465388	14115	14134	ATCTGCTATGCCTGTGAGGA	69	2381
465389	14116	14135	TATCTGCTATGCCTGTGAGG	59	2382
465390	14117	14136	ATATCTGCTATGCCTGTGAG	51	2383
465391	14118	14137	AATATCTGCTATGCCTGTGA	57	2384
465392	14119	14138	GAATATCTGCTATGCCTGTG	60	2385
465393	14120	14139	AGAATATCTGCTATGCCTGT	53	2386
465394	14121	14140	CAGAATATCTGCTATGCCTG	55	2387
465395	14122	14141	TCAGAATATCTGCTATGCCT	64	2388
465396	14123	14142	ATCAGAATATCTGCTATGCC	43	2389
465397	14124	14143	AATCAGAATATCTGCTATGC	37	2390
465398	14125	14144	GAATCAGAATATCTGCTATG	22	2391
465399	14126	14145	TGAATCAGAATATCTGCTAT	33	2392
465400	14127	14146	CTGAATCAGAATATCTGCTA	58	2393
465401	14128	14147	TCTGAATCAGAATATCTGCT	77	2394
455569*	14129	14148	ATCTGAATCAGAATATCTGC	67	1868
465406	14130	14149	CATCTGAATCAGAATATCTG	45	2395
465407	14131	14150	CCATCTGAATCAGAATATCT	47	2396
465408	14132	14151	ACCATCTGAATCAGAATATC	55	2397
465409	14133	14152	GACCATCTGAATCAGAATAT	72	2398
465410	14134	14153	GGACCATCTGAATCAGAATA	70	2399
465411	14135	14154	AGGACCATCTGAATCAGAAT	67	2400
465426	14136	14155	AAGGACCATCTGAATCAGAA	71	2401
465427	14137	14156	CAAGGACCATCTGAATCAGA	73	2402
465428	14138	14157	CCAAGGACCATCTGAATCAG	64	2403
465429	14139	14158	ACCAAGGACCATCTGAATCA	54	2404
465446	14140	14159	GACCAAGGACCATCTGAATC	65	2405
465447	14141	14160	GGACCAAGGACCATCTGAAT	72	2406
465448	14142	14161	AGGACCAAGGACCATCTGAA	68	2407
465449	14143	14162	AAGGACCAAGGACCATCTGA	78	2408

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465450	14144	14163	TAAGGACCAAGGACCATCTG	37	2409
465451	14145	14164	CTAAGGACCAAGGACCATCT	73	2410
465452	14146	14165	ACTAAGGACCAAGGACCATC	65	2411
465453	14147	14166	AACTAAGGACCAAGGACCAT	54	2412
465454	14148	14167	AAACTAAGGACCAAGGACCA	49	2413
465455	14149	14168	CAAATAAGGACCAAGGACC	61	2414
465456	14150	14169	TCAAATAAGGACCAAGGAC	53	2415
465457	14151	14170	CTCAAATAAGGACCAAGGA	59	2416
465534	16802	16821	CAACAGAGTGAAATGTAATG	16	2417
465535	16803	16822	TCAACAGAGTGAAATGTAAT	12	2418
465536	16804	16823	CTCAACAGAGTGAAATGTAA	52	2419
465537	16805	16824	GCTCAACAGAGTGAAATGTA	74	2420
465538	16806	16825	TGCTCAACAGAGTGAAATGT	17	2421
465539	16807	16826	ATGCTCAACAGAGTGAAATG	37	2422
465540	16808	16827	AATGCTCAACAGAGTGAAAT	14	2423
465541	16809	16828	GAATGCTCAACAGAGTGAAA	30	2424
465542	16810	16829	AGAATGCTCAACAGAGTGAA	23	2425
465543	16811	16830	TAGAATGCTCAACAGAGTGA	43	2426
465544	16812	16831	ATAGAATGCTCAACAGAGTG	38	2427
465545	16813	16832	CATAGAATGCTCAACAGAGT	38	2428
465546	16814	16833	CCATAGAATGCTCAACAGAG	56	2429
465547	16815	16834	TCCATAGAATGCTCAACAGA	37	2430
465548	16816	16835	ATCCATAGAATGCTCAACAG	48	2431
465549	16817	16836	AATCCATAGAATGCTCAACA	24	2432
465550	16818	16837	AAATCCATAGAATGCTCAAC	34	2433
465551	16819	16838	AAAATCCATAGAATGCTCAA	30	2434
465552	16820	16839	CAAAATCCATAGAATGCTCA	32	2435
465553	16821	16840	TCAAAATCCATAGAATGCTC	46	2436
465554	16822	16841	GTCAAAATCCATAGAATGCT	57	2437
465555	16823	16842	TGTCAAAATCCATAGAATGC	32	2438
465556	16824	16843	TTGTCAAAATCCATAGAATG	5	2439
465557	16825	16844	TTTGTCAAAATCCATAGAAT	2	2440
465558	16826	16845	ATTTGTCAAAATCCATAGAA	17	2441
465559	16827	16846	CATTTGTCAAAATCCATAGA	17	2442
465560	16828	16847	ACATTTGTCAAAATCCATAG	31	2443
465561	16829	16848	CACATTTGTCAAAATCCATA	43	2444
465562	16830	16849	ACACATTTGTCAAAATCCAT	42	2445
465563	16831	16850	CACACATTTGTCAAAATCCA	56	2446
455581*	16832	16851	TCACACATTTGTCAAAATCC	55	1880
465564	16833	16852	ATCACACATTTGTCAAAATC	34	2447
465565	16834	16853	CATCACACATTTGTCAAAAT	40	2448
465566	16835	16854	TCATCACACATTTGTCAAAA	41	2449

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465567	16836	16855	ATCATCACACATTTGTCAA	37	2450
465568	16837	16856	CATCATCACACATTTGTCAA	44	2451
465569	16838	16857	ACATCATCACACATTTGTCA	60	2452
465570	16839	16858	TACATCATCACACATTTGTC	9	2453
465571	16840	16859	ATACATCATCACACATTTGT	48	2454
465572	16841	16860	TATACATCATCACACATTTG	46	2455
465573	16842	16861	ATATACATCATCACACATTT	28	2456
455582*	16863	16882	TATATAATTGTGTACTGGCA	79	1881
465458	16864	16883	TTATATAATTGTGTACTGGC	83	2457
465459	16865	16884	TTTATATAATTGTGTACTGG	22	2458
465460	16866	16885	TTTTATATAATTGTGTACTG	8	2459
465461	16867	16886	ATTTTATATAATTGTGTACT	0	2460
465462	16868	16887	TATTTTATATAATTGTGTAC	1	2461
465463	16869	16888	CTATTTTATATAATTGTGTA	9	2462
465464	16870	16889	ACTATTTTATATAATTGTGT	0	2463
465465	16871	16890	AACTATTTTATATAATTGTG	7	2464
465466	16872	16891	AAACTATTTTATATAATTGT	13	2465
465606	21187	21206	TAATGAGACTTTAGCACTCT	67	2466
455591*	21188	21207	ATAATGAGACTTTAGCACTC	62	1890
465607	21189	21208	AATAATGAGACTTTAGCACT	41	2467
465608	21190	21209	CAATAATGAGACTTTAGCAC	54	2468
465609	21191	21210	GCAATAATGAGACTTTAGCA	6	2469
465610	21193	21212	CTGCAATAATGAGACTTTAG	77	2470
465611	21194	21213	ACTGCAATAATGAGACTTTA	53	2471
465612	21195	21214	AACTGCAATAATGAGACTTT	39	2472
465266	21638	21657	ATTTGAATAAATGAATGAAA	0	2473
465267	21639	21658	TATTTGAATAAATGAATGAA	0	2474
465268	21640	21659	ATATTTGAATAAATGAATGA	0	2475
465269	21641	21660	AATATTTGAATAAATGAATG	0	2476
465270	21642	21661	AAATATTTGAATAAATGAAT	0	2477
465271	21643	21662	CAAATATTTGAATAAATGAA	0	2478
465272	21644	21663	TCAAATATTTGAATAAATGA	0	2479
465273	21645	21664	CTCAAATATTTGAATAAATG	0	2480
465274	21646	21665	GCTCAAATATTTGAATAAAT	0	2481
465275	21647	21666	TGCTCAAATATTTGAATAAA	6	2482
465276	21648	21667	ATGCTCAAATATTTGAATAA	0	2483
465277	21649	21668	AATGCTCAAATATTTGAATA	0	2484
465278	21650	21669	GAATGCTCAAATATTTGAAT	19	2485
465279	21651	21670	AGAATGCTCAAATATTTGAA	0	2486
465280	21652	21671	CAGAATGCTCAAATATTTGA	5	2487
465281	21653	21672	ACAGAATGCTCAAATATTTG	9	2488
465282	21654	21673	TACAGAATGCTCAAATATTT	1	2489

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465283	21655	21674	CTACAGAATGCTCAAATATT	0	2490
465284	21656	21675	ACTACAGAATGCTCAAATAT	0	2491
465285	21657	21676	AACTACAGAATGCTCAAATA	2	2492
465286	21658	21677	CAACTACAGAATGCTCAAAT	12	2493
465287	21659	21678	GCAACTACAGAATGCTCAAA	26	2494
465288	21660	21679	AGCAACTACAGAATGCTCAA	39	2495
465289	21661	21680	CAGCAACTACAGAATGCTCA	53	2496
465290	21662	21681	CCAGCAACTACAGAATGCTC	26	2497
465291	21663	21682	CCCAGCAACTACAGAATGCT	42	2498
465292	21664	21683	CCCCAGCAACTACAGAATGC	40	2499
465293	21665	21684	TCCCCAGCAACTACAGAATG	13	2500
465294	21666	21685	TTCCCCAGCAACTACAGAAT	30	2501
465295	21667	21686	TTTCCCCAGCAACTACAGAA	16	2502
465296	21668	21687	ATTCCCCAGCAACTACAGA	5	2503
465297	21669	21688	TATTTCCCCAGCAACTACAG	7	2504
465298	21670	21689	CTATTTCCCCAGCAACTACA	20	2505
465299	21671	21690	GCTATTTCCCCAGCAACTAC	7	2506
465300	21672	21691	TGCTATTTCCCCAGCAACTA	25	2507
465301	21673	21692	CTGCTATTTCCCCAGCAACT	31	2508
465302	21674	21693	ACTGCTATTTCCCCAGCAAC	14	2509
455594*	21675	21694	CACTGCTATTTCCCCAGCAA	43	1893
465303	21676	21695	TCACTGCTATTTCCCCAGCA	23	2510
465304	21677	21696	TTCCTGCTATTTCCCCAGC	45	2511
465305	21678	21697	GTTCACTGCTATTTCCCCAG	11	2512
465306	21679	21698	AGTTCCTGCTATTTCCCCA	62	2513
465307	21680	21699	CAGTTCCTGCTATTTCCCC	52	2514
465308	21681	21700	TCAGTTCCTGCTATTTCCC	40	2515
465309	21682	21701	TTCAGTTCCTGCTATTTCC	29	2516
465310	21683	21702	CTTCAGTTCCTGCTATTTTC	40	2517
465311	21684	21703	TCTTCAGTTCCTGCTATTT	25	2518
465312	21685	21704	TTCTTCAGTTCCTGCTATT	18	2519
465313	21686	21705	ATTCTTCAGTTCCTGCTAT	7	2520
465314	21687	21706	CATTCTTCAGTTCCTGCTA	33	2521
465315	21688	21707	ACATTCTTCAGTTCCTGCT	39	2522
465316	21689	21708	GACATTCTTCAGTTCCTGCT	49	2523
465317	21690	21709	AGACATTCTTCAGTTCCTG	50	2524
465318	21691	21710	AAGACATTCTTCAGTTCCT	37	2525
465319	21692	21711	AAAGACATTCTTCAGTTCAC	26	2526
465320	21693	21712	CAAAGACATTCTTCAGTTCA	13	2527
465321	21694	21713	ACAAAGACATTCTTCAGTTC	0	2528
465322	21695	21714	AACAAAGACATTCTTCAGTT	11	2529
465323	21696	21715	GAACAAAGACATTCTTCAGT	10	2530

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465324	21697	21716	AGAACAAAGACATTCTTCAG	14	2531
465325	21698	21717	AAGAACAAAGACATTCTTCA	7	2532
465326	21699	21718	TAAGAACAAAGACATTCTTC	13	2533
465327	21700	21719	ATAAGAACAAAGACATTCTT	1	2534
465328	21701	21720	CATAAGAACAAAGACATTCT	16	2535
465329	21702	21721	CCATAAGAACAAAGACATTC	38	2536
465330	21703	21722	CCCATAAGAACAAAGACATT	11	2537
465331	21704	21723	CCCCATAAGAACAAAGACAT	0	2538
465332	21705	21724	GCCCCATAAGAACAAAGACA	30	2539
465333	21706	21725	AGCCCCATAAGAACAAAGAC	22	2540
465334	21707	21726	AAGCCCCATAAGAACAAAGA	21	2541
465613	26034	26053	TCTCCAGCCTACAGATGACT	32	2542
465614	26035	26054	CTCTCCAGCCTACAGATGAC	31	2543
465615	26036	26055	TCTCTCCAGCCTACAGATGA	29	2544
465616	26037	26056	CTCTCTCCAGCCTACAGATG	22	2545
465617	26038	26057	CCTCTCTCCAGCCTACAGAT	44	2546
465618	26039	26058	TCCTCTCTCCAGCCTACAGA	41	2547
465619	26040	26059	TTCTCTCTCCAGCCTACAG	32	2548
465620	26041	26060	GTTCTCTCTCCAGCCTACA	0	2549
465621	26042	26061	AGTTCTCTCTCCAGCCTAC	44	2550
465622	26043	26062	CAGTTCTCTCTCCAGCCTA	39	2551
465623	26044	26063	CCAGTTCTCTCTCCAGCCT	47	2552
465624	26045	26064	TCCAGTTCTCTCTCCAGCC	49	2553
465625	26046	26065	TTCCAGTTCTCTCTCCAGC	46	2554
465626	26047	26066	CTTCCAGTTCTCTCTCCAG	47	2555
465627	26048	26067	CCTTCCAGTTCTCTCTCCA	28	2556
465628	26049	26068	CCCTTCCAGTTCTCTCTCC	28	2557
465629	26050	26069	CCCCTTCCAGTTCTCTCTC	21	2558
465630	26051	26070	GCCCCTTCCAGTTCTCTCT	65	2559
465631	26052	26071	AGCCCCTTCCAGTTCTCTCT	60	2560
465632	26053	26072	TAGCCCCTTCCAGTTCTCT	56	2561
465633	26054	26073	TTAGCCCCTTCCAGTTCTCT	52	2562
465634	26055	26074	TTTAGCCCCTTCCAGTTCT	53	2563
465635	26056	26075	CTTTAGCCCCTTCCAGTTCC	39	2564
465636	26057	26076	ACTTTAGCCCCTTCCAGTTC	31	2565
465637	26058	26077	AACTTTAGCCCCTTCCAGTT	46	2566
465638	26059	26078	CAACTTTAGCCCCTTCCAGT	37	2567
465639	26060	26079	CCAACTTTAGCCCCTTCCAG	48	2568
455611*	26061	26080	GCCAACTTTAGCCCCTTCCA	62	1870
465640	26062	26081	AGCCAACTTTAGCCCCTTCC	71	2569
465641	26063	26082	CAGCCAACTTTAGCCCCTTC	70	2570
465642	26064	26083	TCAGCCAACTTTAGCCCCTT	66	2571

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465643	26065	26084	CTCAGCCAACTTTAGCCCCT	35	2572
465644	26066	26085	ACTCAGCCAACTTTAGCCCC	49	2573
465645	26067	26086	TACTCAGCCAACTTTAGCCC	33	2574
465646	26068	26087	CTACTCAGCCAACTTTAGCC	28	2575
465647	26069	26088	ACTACTCAGCCAACTTTAGC	12	2576
465648	26070	26089	AACTACTCAGCCAACTTTAG	34	2577
465649	26071	26090	TAACTACTCAGCCAACTTTA	26	2578
455637*	37873	37892	GTACTTTACATGTGCAGCAC	78	1931
465650	37874	37893	TGTACTTTACATGTGCAGCA	71	2579
465651	37875	37894	GTGTACTTTACATGTGCAGC	75	2580
465652	37876	37895	TGTGTACTTTACATGTGCAG	65	2581
465653	37877	37896	CTGTGTACTTTACATGTGCA	65	2582
465654	37878	37897	CCTGTGTACTTTACATGTGC	60	2583
465655	37879	37898	TCCTGTGTACTTTACATGTG	51	2584
465656	37880	37899	CTCCTGTGTACTTTACATGT	48	2585
465657	37881	37900	TCTCCTGTGTACTTTACATG	25	2586
465658	37882	37901	ATCTCCTGTGTACTTTACAT	33	2587
465659	37883	37902	AATCTCCTGTGTACTTTACA	23	2588
465660	37884	37903	AAATCTCCTGTGTACTTTAC	24	2589
465661	37885	37904	TAAATCTCCTGTGTACTTTA	26	2590
465666	37886	37905	CTAAATCTCCTGTGTACTTT	16	2591
465667	37887	37906	TCTAAATCTCCTGTGTACTT	27	2592
465668	37888	37907	TTCTAAATCTCCTGTGTACT	30	2593
465669	37889	37908	TTTCTAAATCTCCTGTGTAC	30	2594
465670	37890	37909	TTTTCTAAATCTCCTGTGTA	11	2595
465671	37891	37910	GTTTTCTAAATCTCCTGTGT	37	2596
465672	37892	37911	AGTTTTCTAAATCTCCTGTG	49	2597
465686	37893	37912	AAGTTTTCTAAATCTCCTGT	19	2598
465687	37894	37913	GAAGTTTTCTAAATCTCCTG	46	2599
465688	37895	37914	CGAAGTTTTCTAAATCTCCT	53	2600
465689	37896	37915	ACGAAGTTTTCTAAATCTCC	45	2601
465690	37897	37916	TACGAAGTTTTCTAAATCTC	9	2602
465706	37898	37917	CTACGAAGTTTTCTAAATCT	14	2603
465707	37899	37918	GCTACGAAGTTTTCTAAATC	32	2604
455677*	45512	45531	TTCCAATATTTGTACCCTCA	49	1971
465574	45513	45532	TTTCCAATATTTGTACCCTC	43	2605
465575	45514	45533	CTTTCCAATATTTGTACCCT	50	2606
465576	45515	45534	GCTTTCCAATATTTGTACCC	58	2607
465577	45516	45535	TGCTTTCCAATATTTGTACC	35	2608
465578	45517	45536	TTGCTTTCCAATATTTGTAC	31	2609
465579	45518	45537	CTTGCTTTCCAATATTTGTA	29	2610
465580	45519	45538	CCTTGCTTTCCAATATTTGT	35	2611

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465581	45520	45539	CCCTTGCTTTCCAATATTG	26	2612
465582	45521	45540	TCCCTTGCTTTCCAATATTT	34	2613
465583	45522	45541	GTCCCTTGCTTTCCAATATT	39	2614
465584	45523	45542	TGTCCCTTGCTTTCCAATAT	44	2615
465585	45524	45543	CTGTCCCTTGCTTTCCAATA	60	2616
465586	45525	45544	TCTGTCCCTTGCTTTCCAAT	59	2617
465587	45526	45545	TTCTGTCCCTTGCTTTCCAA	47	2618
455681*	46091	46110	TTTCCAGATATTTTCCCATA	48	1975
465335	46092	46111	GTTTCCAGATATTTTCCCAT	71	2619
465336	46093	46112	TGTTTCCAGATATTTTCCCA	53	2620
466676	48396	48415	CTTTCATTCTAGTTTTACC	1	2621
466677	48397	48416	ACTTTCATTCTAGTTTTAC	19	2622
466678	48398	48417	CAC TTTCCATTCTAGTTT A	23	2623
466679	48399	48418	ACACTTTCATTCTAGTTTT	9	2624
466680	48400	48419	CACACTTTCATTCTAGTTT	31	2625
466681	48401	48420	CCACACTTTCATTCTAGTT	64	2626
455703*	48402	48421	GCCACACTTTCATTCTAGT	75	1997
466682	48403	48422	AGCCACACTTTCATTCTAG	56	2627
466683	48404	48423	AAGCCACACTTTCATTCTA	40	2628
466684	48405	48424	CAAGCCACACTTTCATTCT	24	2629
466685	48406	48425	TCAAGCCACACTTTCATTCT	39	2630
466686	48407	48426	CTCAAGCCACACTTTCATT	38	2631
466687	48408	48427	GCTCAAGCCACACTTTCAT	53	2632
466688	48409	48428	AGCTCAAGCCACACTTTCCA	59	2633
466689	48410	48429	CAGCTCAAGCCACACTTTCC	51	2634
466690	48411	48430	CCAGCTCAAGCCACACTTTC	43	2635
466691	48412	48431	ACCAGCTCAAGCCACACTTT	30	2636
466692	48413	48432	TACCAGCTCAAGCCACACTT	35	2637
466693	48414	48433	TTACCAGCTCAAGCCACACT	32	2638
466694	48415	48434	GTTACCAGCTCAAGCCACAC	53	2639
466695	48416	48435	GGTTACCAGCTCAAGCCACA	54	2640
455704*	48417	48436	TGGTTACCAGCTCAAGCCAC	61	1998
455708*	48728	48747	CCCACAGTGACAGTGACTCA	58	2002
465708	48729	48748	TCCCACAGTGACAGTGACTC	61	2641
465709	48730	48749	TTCCCACAGTGACAGTGACT	60	2642
465710	48731	48750	CTTCCCACAGTGACAGTGAC	55	2643
455723*	52033	52052	ACCAGTTTTCTAGCCGATCT	24	2017
466696	52034	52053	TACCAGTTTTCTAGCCGATC	54	2644
466697	52035	52054	TTACCAGTTTTCTAGCCGAT	41	2645
466698	52036	52055	TTTACCAGTTTTCTAGCCGA	37	2646
466699	52037	52056	CTTTACCAGTTTTCTAGCCG	17	2647
466700	52038	52057	CCTTTACCAGTTTTCTAGCC	11	2648

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466701	52039	52058	TCCTTTACCAGTTTCTAGC	24	2649
466702	52040	52059	ATCCTTTACCAGTTTCTAG	1	2650
466703	52041	52060	CATCCTTTACCAGTTTCTA	7	2651
466704	52042	52061	TCATCCTTTACCAGTTTCT	0	2652
466705	52043	52062	TTCATCCTTTACCAGTTTC	15	2653
466706	52044	52063	TTTCATCCTTTACCAGTTT	0	2654
466707	52045	52064	CTTTCATCCTTTACCAGTT	9	2655
466708	52046	52065	TCTTTCATCCTTTACCAGT	0	2656
466709	52047	52066	TTCTTTCATCCTTTACCAGT	8	2657
466710	52048	52067	CTTCTTTCATCCTTTACCAG	11	2658
466711	52049	52068	GCTTCTTTCATCCTTTACCA	8	2659
466712	52050	52069	AGCTTCTTTCATCCTTTACC	6	2660
466713	52051	52070	AAGCTTCTTTCATCCTTTAC	0	2661
466714	52052	52071	AAAGCTTCTTTCATCCTTTA	18	2662
466715	52053	52072	AAAAGCTTCTTTCATCCTTT	2	2663
466716	52054	52073	GAAAAGCTTCTTTCATCCTT	9	2664
466717	52055	52074	GGAAAAGCTTCTTTCATCCT	1	2665
455724*	52056	52075	AGGAAAAGCTTCTTTCATCC	0	2018
455762*	59913	59932	CCAAGTGTTTGAATTCTGCA	36	2056
466766	59914	59933	ACCAAGTGTTTGAATTCTGC	58	2666
466767	59915	59934	TACCAAGTGTTTGAATTCTG	32	2667
466768	59916	59935	ATACCAAGTGTTTGAATTCT	21	2668
466769	59917	59936	CATACCAAGTGTTTGAATTC	9	2669
466770	59918	59937	ACATACCAAGTGTTTGAATT	14	2670
466771	59919	59938	CACATACCAAGTGTTTGAAT	26	2671
466772	59920	59939	CCACATACCAAGTGTTTGAA	8	2672
466773	59921	59940	CCCACATACCAAGTGTTTGA	19	2673
466774	59922	59941	TCCCACATACCAAGTGTTTG	5	2674
466775	59923	59942	CTCCCACATACCAAGTGTTT	25	2675
466776	59924	59943	CCTCCCACATACCAAGTGTT	32	2676
466777	59925	59944	TCCTCCCACATACCAAGTGT	12	2677
466778	59926	59945	CTCCTCCCACATACCAAGTG	10	2678
466779	59927	59946	GCTCCTCCCACATACCAAGT	15	2679
466780	59928	59947	AGCTCCTCCCACATACCAAG	5	2680
466781	59929	59948	GAGCTCCTCCCACATACCAA	23	2681
465768	61325	61344	CAGTCTAGAATAGCCATGGA	71	2682
465769	61326	61345	ACAGTCTAGAATAGCCATGG	72	2683
465770	61327	61346	GACAGTCTAGAATAGCCATG	78	2684
465771	61328	61347	AGACAGTCTAGAATAGCCAT	74	2685
465772	61329	61348	GAGACAGTCTAGAATAGCCA	70	2686
465773	61330	61349	AGAGACAGTCTAGAATAGCC	70	2687
465774	61331	61350	CAGAGACAGTCTAGAATAGC	63	2688

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465775	61332	61351	ACAGAGACAGTCTAGAATAG	55	2689
465776	61333	61352	CACAGAGACAGTCTAGAATA	64	2690
465777	61334	61353	TCACAGAGACAGTCTAGAAT	71	2691
465778	61335	61354	ATCACAGAGACAGTCTAGAA	79	2692
465779	61336	61355	TATCACAGAGACAGTCTAGA	66	2693
465780	61337	61356	ATATCACAGAGACAGTCTAG	64	2694
465781	61338	61357	AATATCACAGAGACAGTCTA	48	2695
465782	61339	61358	AAATATCACAGAGACAGTCT	65	2696
455786*	61340	61359	CAAATATCACAGAGACAGTC	63	2070
465783	61341	61360	GCAAATATCACAGAGACAGT	69	2697
465786	61342	61361	TGCAAATATCACAGAGACAG	78	2698
465787	61343	61362	ATGCAAATATCACAGAGACA	72	2699
465788	61344	61363	AATGCAAATATCACAGAGAC	59	2700
465789	61345	61364	AAATGCAAATATCACAGAGA	23	2701
465790	61346	61365	AAAATGCAAATATCACAGAG	28	2702
465791	61347	61366	TAAAATGCAAATATCACAGA	0	2703
465792	61348	61367	TTAAAATGCAAATATCACAG	12	2704
465793	61349	61368	TTTAAAATGCAAATATCACA	3	2705
465794	61350	61369	GTTTAAAATGCAAATATCAC	2	2706
465795	61351	61370	AGTTTAAAATGCAAATATCA	0	2707
465796	61352	61371	CAGTTTAAAATGCAAATATC	13	2708
465797	61353	61372	TCAGTTTAAAATGCAAATAT	0	2709
465798	61354	61373	TTCAGTTTAAAATGCAAATA	0	2710
465799	61355	61374	ATTCAGTTTAAAATGCAAAT	1	2711
465800	61356	61375	TATTCAGTTTAAAATGCAAA	0	2712
465801	61357	61376	ATATTCAGTTTAAAATGCAA	0	2713
455790*	62043	62062	CATGGTTATGTGTATCTGCA	69	2074
465337	62044	62063	ACATGGTTATGTGTATCTGC	69	2714
465338	62045	62064	CACATGGTTATGTGTATCTG	40	2715
465339	62046	62065	CCACATGGTTATGTGTATCT	32	2716
337332	66135	66154	GAAGCCCTTGCCAGCCATGT	79	1541
455840*	71610	71629	GTACAATTGCTTCAACTAGA	81	2124
466782	71611	71630	AGTACAATTGCTTCAACTAG	54	2717
466783	71612	71631	CAGTACAATTGCTTCAACTA	68	2718
466784	71613	71632	GCAGTACAATTGCTTCAACT	72	2719
465588	71614	71633	GGCAGTACAATTGCTTCAAC	69	2720
455264*	74768	74787	TCCTTAAACCTTCCTATTTT	26	1563
465226	74769	74788	CTCCTTAAACCTTCCTATTT	45	2721
455265*	74770	74789	TCTCCTTAAACCTTCCTATT	57	1564
465227	74771	74790	TTCTCCTTAAACCTTCCTAT	54	2722
455266*	74772	74791	ATTCTCCTTAAACCTTCCTA	52	1565
465228	74773	74792	GATTCTCCTTAAACCTTCCT	64	2723

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455267*	74774	74793	AGATTCTCCTTAAACCTTCC	60	1566
465229	74775	74794	TAGATTCTCCTTAAACCTTC	22	2724
455268*	74776	74795	TTAGATTCTCCTTAAACCTT	55	1567
465230	74777	74796	CTTAGATTCTCCTTAAACCT	69	2725
455269*	74778	74797	GCTTAGATTCTCCTTAAACC	84	1568
465231	74779	74798	TGCTTAGATTCTCCTTAAAC	64	2726
455270*	74780	74799	ATGCTTAGATTCTCCTTAAA	50	1569
465232	74781	74800	AATGCTTAGATTCTCCTTAA	71	2727
455271*	74782	74801	AAATGCTTAGATTCTCCTTA	69	1570
465233	74783	74802	AAAATGCTTAGATTCTCCTT	69	2728
455272*	74784	74803	TAAAATGCTTAGATTCTCCT	56	1571
455281*	74872	74891	CAAGGTTGTAAGCACCTCT	63	1580
465234	74873	74892	TCAAGGTTGTAAGCACCTC	54	2729
455282*	74874	74893	GTCAAGGTTGTAAGCACCT	8	1581
465235	74875	74894	AGTCAAGGTTGTAAGCACCC	65	2730
455283*	74876	74895	GAGTCAAGGTTGTAAGCAC	48	1582
455290*	74900	74919	GCAGATCAAGTCCAGGGAGA	77	1589
465236	74901	74920	AGCAGATCAAGTCCAGGGAG	80	2731
455291*	74902	74921	CAGCAGATCAAGTCCAGGGA	82	1590
465237	74903	74922	ACAGCAGATCAAGTCCAGGG	82	2732
455292*	74904	74923	AACAGCAGATCAAGTCCAGG	69	1591
455369*	75418	75437	GGTGTTCACATACGCACAGG	75	1668
465238	75419	75438	AGGTGTTCACATACGCACAG	68	2733
455370*	75420	75439	TAGGTGTTCACATACGCACA	67	1669
465239	75421	75440	CTAGGTGTTCACATACGCAC	82	2734
455371*	75422	75441	GCTAGGTGTTCACATACGCA	85	1670
465240	75423	75442	TGCTAGGTGTTCACATACGC	77	2735
455372*	75424	75443	GTGCTAGGTGTTCACATACG	72	1671
455390*	75616	75635	AACTGTCTCCAGGCAGGAGG	65	1689
465241	75617	75636	CAACTGTCTCCAGGCAGGAG	51	2736
455391*	75618	75637	TCAACTGTCTCCAGGCAGGA	52	1690
465242	75619	75638	ATCAACTGTCTCCAGGCAGG	76	2737
455392*	75620	75639	CATCAACTGTCTCCAGGCAG	63	1691
465243	75621	75640	ACATCAACTGTCTCCAGGCA	70	2738
455393*	75622	75641	CACATCAACTGTCTCCAGGC	75	1692
465244	75623	75642	ACACATCAACTGTCTCCAGG	61	2739
455394*	75624	75643	GACACATCAACTGTCTCCAG	69	1693
455397*	75662	75681	TACTGAAGAGTGTTGCTGGA	77	1696
465245	75663	75682	GTACTGAAGAGTGTTGCTGG	84	2740
455398*	75664	75683	TGTACTGAAGAGTGTTGCTG	76	1697
465246	75665	75684	ATGTACTGAAGAGTGTTGCT	72	2741
455399*	75666	75685	TATGTACTGAAGAGTGTTGC	70	1698

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455411*	75726	75745	AACCCAATGGTAAGCCCAAG	77	1710
465247	75727	75746	AAACCCAATGGTAAGCCCAA	61	2742
455412*	75728	75747	TAAACCCAATGGTAAGCCCA	72	1711
465248	75729	75748	TTAAACCCAATGGTAAGCCC	69	2743
455413*	75730	75749	TTTAAACCCAATGGTAAGCC	38	1712
455428*	75829	75848	TACAATCAGAGTTAAGACCA	58	1727
465249	75830	75849	CTACAATCAGAGTTAAGACC	58	2744
455429*	75831	75850	GCTACAATCAGAGTTAAGAC	71	1728
465250	75832	75851	TGCTACAATCAGAGTTAAGA	59	2745
455430*	75833	75852	TTGCTACAATCAGAGTTAAG	47	1729
455437*	75847	75866	TCCTCTCAGAACTTTTGCTA	36	1736
465251	75848	75867	CTCCTCTCAGAACTTTTGCT	47	2746
455438*	75849	75868	GCTCCTCTCAGAACTTTTGC	75	1737
465252	75850	75869	AGCTCCTCTCAGAACTTTTG	71	2747
455439*	75851	75870	CAGCTCCTCTCAGAACTTTT	68	1738
465253	75852	75871	TCAGCTCCTCTCAGAACTTT	62	2748
455440*	75853	75872	CTCAGCTCCTCTCAGAACTT	58	1739
455446*	75965	75984	GTAGGTAAGCAACCCACGGG	69	1745
465254	75966	75985	GGTAGGTAAGCAACCCACGG	79	2749
455447*	75967	75986	AGGTAGGTAAGCAACCCACG	80	1476
465255	75968	75987	TAGGTAGGTAAGCAACCCAC	84	2750
455448*	75969	75988	ATAGGTAGGTAAGCAACCCA	71	1474
455456*	75985	76004	GCTTATAAACCACCTTATAG	37	1755
465256	75986	76005	AGCTTATAAACCACCTTATA	43	2751
455457*	75987	76006	CAGCTTATAAACCACCTTAT	57	1756
465257	75988	76007	GCAGCTTATAAACCACCTTA	73	2752
455458*	75989	76008	AGCAGCTTATAAACCACCTT	75	1757
465258	75990	76009	CAGCAGCTTATAAACCACCT	65	2753
455459*	75991	76010	ACAGCAGCTTATAAACCACC	46	1758
455462*	75997	76016	GCCAGGACAGCAGCTTATAA	70	1761
466718	75998	76017	GGCCAGGACAGCAGCTTATA	87	2754
455463*	75999	76018	TGGCCAGGACAGCAGCTTAT	83	1762
466719	76000	76019	GTGGCCAGGACAGCAGCTTA	76	2755
455464*	76001	76020	AGTGGCCAGGACAGCAGCTT	82	1763
455470*	76013	76032	GAATTTGAATGCAGTGGCCA	75	1769
466720	76014	76033	GGAATTTGAATGCAGTGGCC	87	2756
455471*	76015	76034	TGGAATTTGAATGCAGTGGC	75	1770
466721	76016	76035	TTGGAATTTGAATGCAGTGG	72	2757
455472*	76017	76036	ATTGGAATTTGAATGCAGTG	60	1771

Example 35: Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

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Gapmers from the study described in Example 3 exhibiting significant *in vitro* inhibition of STAT3 were tested at various doses in HuVEC cells. Cells were plated at a density of 5,000 cells per well and transfected using LipofectAMINE2000® reagent with 8.8 nM, 17.5 nM, 35.0 nM, and 70.0 nM concentrations of antisense oligonucleotide, as specified in Table 56. After a treatment period of approximately 16 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

As illustrated in Table 56, STAT3 mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 56

Dose-dependent antisense inhibition of human STAT3 in HuVEC cells using LipofectAMINE 2000® reagent

ISIS No	8.8 nM	17.5 nM	35.0 nM	70.0 nM
337332	50	71	81	88
455269	62	69	79	82
455291	72	81	87	88
455371	71	83	88	90
455447	53	70	81	79
455463	68	79	84	87
455464	69	78	84	86
455471	62	82	88	90
455547	43	64	75	87
455565	41	73	83	92
455582	50	67	81	87
455637	50	65	79	85
455703	45	65	81	85
455840	58	70	80	85
465236	62	76	81	85
465237	67	81	86	90
465239	64	77	85	92
465240	50	66	76	83
465245	70	81	87	87
465254	54	75	81	86
465255	63	74	84	85
465335	46	62	74	80
465449	49	71	84	84
465458	54	73	84	88
465509	66	80	86	83
465510	48	66	76	82

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465511	56	68	75	79
465526	53	68	76	76
465537	41	60	77	85
465588	52	73	76	79
465610	35	57	71	79
465730	51	75	85	87
465739	72	81	88	90
465740	70	81	86	89
465742	63	76	87	88
465748	48	62	67	74
465751	70	81	87	87
465752	76	82	88	89
465754	70	83	86	87
465755	70	81	85	89
465770	52	69	77	77
465771	40	55	64	75
465778	40	69	75	77
465786	56	71	76	83
465830	66	77	83	82
465833	50	67	79	86
465834	42	67	77	81
465886	58	73	83	87
465888	49	68	82	12
465926	43	64	76	82
466661	47	63	80	84
466666	39	66	80	86
466670	73	83	89	90
466718	73	78	84	85
466719	63	73	83	83
466720	80	87	86	86

Example 36: Dose-dependent antisense inhibition of human STAT3 in HuVEC cells

Gapmers from the study described in Example 3 were further tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 187.5 nM, 375.0 nM, 750.0 nM, 1,500.0 nM, 3,000.0 nM, and 6,000.0 nM concentrations of antisense oligonucleotide, as specified in Table 57. After a treatment period of approximately 16 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

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As illustrated in Table 57, STAT3 mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 57
Dose-dependent antisense inhibition of human STAT3 in HuVEC cells using electroporation

ISIS No	187.5 nM	375.0 nM	750.0 nM	1500.0 nM	3000.0 nM	6000.0 nM	IC ₅₀ (μ M)
337332	35	51	73	84	97	98	0.3
455269	64	76	87	89	92	90	<0.2
455291	63	79	88	90	90	93	<0.2
455371	50	81	90	94	96	95	<0.2
455447	37	49	61	91	94	96	0.3
455463	57	78	89	93	95	94	<0.2
455464	57	67	78	80	79	87	<0.2
455471	50	73	81	86	91	92	<0.2
455547	19	49	63	82	92	94	0.5
455582	42	62	82	92	97	97	0.2
455637	44	60	63	87	91	92	0.2
455840	39	58	75	81	88	89	0.2
465236	56	67	71	83	91	92	<0.2
465237	56	75	87	92	94	93	<0.2
465239	60	78	88	95	99	99	<0.2
465240	49	67	80	85	94	95	0.1
465245	54	67	81	86	90	90	<0.2
465254	28	50	63	76	91	92	0.4
465255	46	55	78	89	92	94	0.2
465335	25	52	65	89	95	95	0.4
465449	28	56	78	72	96	96	0.3
465458	19	68	84	91	96	97	0.3
465509	42	68	77	84	88	88	0.1
465510	15	43	60	73	85	88	0.6
465511	19	39	47	68	79	86	0.8
465526	15	39	54	64	82	84	0.8
465537	44	65	82	90	95	90	0.1
465565	12	45	62	80	93	97	0.6
465588	44	66	82	85	85	87	0.1
465610	33	56	72	89	96	97	0.3
465730	48	51	72	91	94	91	0.2
465739	42	78	85	93	96	92	0.9
465740	54	69	80	96	98	98	<0.2
465742	67	55	91	93	87	93	<0.2
465748	49	67	88	96	98	99	0.1

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465751	56	63	82	91	98	98	0.1
465752	62	79	84	93	96	90	<0.2
465754	41	69	84	63	94	93	<0.2
465755	47	56	67	83	93	97	0.2
465770	52	54	70	85	88	83	0.2
465771	38	62	76	83	84	86	0.2
465778	40	58	79	84	96	96	0.2
465786	41	68	88	94	95	93	0.1
465830	50	73	89	93	88	92	<0.2
465833	27	44	76	89	88	97	0.4
465834	8	27	57	80	93	97	0.7
465886	58	79	90	97	98	96	<0.2
465888	39	60	65	90	94	97	0.3
465926	23	50	41	85	93	94	0.5
466661	31	58	76	90	95	96	0.3
466666	44	55	79	92	96	97	0.2
466670	50	54	82	96	96	96	0.2
466718	55	79	90	93	95	96	<0.2
466719	44	52	73	65	87	91	0.3
466720	48	78	90	90	90	90	<0.2

Example 37: Tolerability of antisense oligonucleotides targeting human STAT3 in CD1 mice

Thirty-nine antisense oligonucleotides exhibiting a high level of potency were further tested for in vivo tolerability.

5 Groups of eight male CD1 mice were injected subcutaneously twice a week for 6 weeks with 50 mg/kg of ISIS antisense oligonucleotides. One group of eight male CD1 mice was injected subcutaneously twice a week for 6 weeks with PBS. This group served as the control group. Three days after the last dose mice were euthanized and organs and plasma were harvested for further analysis. Liver, spleen, and kidney weights were measured at the end of the study and were compared to PBS treated mice.

10 To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured.

15 To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

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Blood obtained from all mice groups were sent to Antech Diagnostics for hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) measurements and analyses, as well as measurements of the differential blood cell counts, such as that of WBC, RBC, and total hemoglobin content.

Among the 39 antisense oligonucleotides tested, certain antisense oligonucleotides, including ISIS 455265, ISIS 455269, ISIS 455271, ISIS 455272, ISIS 455291, ISIS 455371, ISIS 455394, ISIS 455703, ISIS 455429, ISIS 455471, ISIS 455527, ISIS 455530, ISIS 455536, ISIS 455548, ISIS 455611, ISIS 465236, ISIS 465237, ISIS 465588, ISIS 465740, ISIS 465754, ISIS 465830, ISIS 466670, and ISIS 466720 met tolerability thresholds for organ weight, ALT, AST, BUN, and hematological parameters.

Example 38: Measurement of half-life of antisense oligonucleotide in CD1 mouse liver

CD1 mice were treated with ISIS antisense oligonucleotides described and the oligonucleotide half-life in the liver was evaluated.

Treatment

Groups of twelve CD1 mice each were injected subcutaneously twice per week for 2 weeks with 50 mg/kg of ISIS 455265, ISIS 455269, ISIS 455271, ISIS 455272, ISIS 455291, ISIS 455371, ISIS 455393, ISIS 455553, ISIS 455582, ISIS 455703, ISIS 455394, ISIS 455429, ISIS 455438, ISIS 455471, ISIS 455527, ISIS 455530, ISIS 455536, ISIS 455540, ISIS 455548, ISIS 455611, ISIS 455429, ISIS 455463, ISIS 455464, ISIS 455471, ISIS 455527, ISIS 455611, ISIS 465236, ISIS 465237, ISIS 465239, ISIS 465588, ISIS 465740, ISIS 465742, ISIS 465751, ISIS 465752, ISIS 465754, ISIS 465830, ISIS 466670, ISIS 466718, and ISIS 466720. Four mice from each group were sacrificed 3 days, 28 days, and 56 days following the final dose. Livers were harvested for analysis.

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999), which includes a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTT, designated herein as SEQ ID NO: 2758) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 µg/g. Half-lives were then calculated using WinNonlin software (PHARSIGHT).

The half-life of each oligonucleotide is presented in Table 58. Antisense oligonucleotides with half-lives within 11-34 days were chosen for further studies.

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Table 58
Half-life of ISIS oligonucleotides in the liver of CD1 mice

ISIS No	Half-life (days)
455265	12
455269	48
455271	16
455272	16
455291	19
455371	28
455394	17
455703	27
455429	15
455471	15
455527	13
455530	12
455536	20
455548	13
455611	37
465236	22
465237	17
465588	14
465740	15
465754	23
465830	23
466670	11
466720	17

5 Example 39: Tolerability of antisense oligonucleotides targeting human STAT3 in Sprague-Dawley rats

Twenty-three antisense oligonucleotides exhibiting a high level of potency were further tested for in vivo tolerability.

Groups of four Sprague-Dawley rats were injected subcutaneously twice a week for 6 weeks with 50 mg/kg of ISIS antisense oligonucleotides. One group of rats was injected subcutaneously twice a week for 6 weeks with PBS. This group served as the control group. Three days after the last dose rats were euthanized and organs and plasma were harvested for further analysis. Liver, spleen, and kidney weights were measured at the end of the study and were compared to PBS treated rats

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To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of AST (aspartate transaminase) and total bilirubin were measured.

To evaluate the effect of ISIS oligonucleotides on kidney function, BUN, total urine protein, and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

Among the 23 antisense oligonucleotides tested, certain antisense oligonucleotides, including ISIS 455269, ISIS 455291, ISIS 455371, ISIS 455703, ISIS 455429, ISIS 465236, ISIS 465237, ISIS 465754, ISIS 465830, and ISIS 466670 met tolerability thresholds for organ weight, AST, bilirubin, BUN, total urine protein, and creatinine.

Example 40: Measurement of half-life of antisense oligonucleotide in Sprague-Dawley rat liver and kidney

Sprague Dawley rats were treated with ISIS antisense oligonucleotides and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver and kidney was evaluated.

Treatment

Groups of four Sprague Dawley rats each were injected subcutaneously twice a week for 2 weeks with 20 mg/kg of ISIS 455265, ISIS 455269, ISIS 455271, ISIS 455272, ISIS 455291, ISIS 455371, ISIS 455394, ISIS 455703, ISIS 455429, ISIS 455471, ISIS 455527, ISIS 455530, ISIS 455536, ISIS 455548, ISIS 455611, ISIS 465236, ISIS 465237, ISIS 465588, ISIS 465740, ISIS 465754, ISIS 465830, ISIS 466670, and ISIS 466720. Three days after the last dose, the rats were sacrificed and livers and kidneys were collected for analysis.

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999), which includes a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTT, designated herein as SEQ ID NO: 2758) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 µg/g. The kidney to liver ratio of the full-length oligonucleotide concentration, as well as that for the total oligonucleotide concentration were calculated. The results are presented in Table 59.

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Table 59

Kidney to liver ratio of full-length and total oligonucleotide concentrations in Sprague-Dawley rats

ISIS No	Full length	Total
455265	3.6	3.8
455269	2.1	2.4
455271	3.1	3.0
455272	2.9	3.1
455291	2.7	3.3
455371	2.2	2.4
455394	1.8	2.2
455703	2.3	2.8
455429	3.8	3.9
455471	2.7	2.9
455527	5.0	3.9
455530	3.9	2.9
455536	3.5	3.6
455548	2.5	2.9
455611	2.3	2.3
465236	2.3	3.3
465237	2.4	2.7
465588	2.8	2.6
465740	2.4	2.6
465754	1.6	1.8
465830	5.1	2.6
466670	3.1	4.4
466720	2.3	2.6

Example 41: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in SK-BR-3 cells

Gapmers from the rodent tolerability studies described in Examples 6-9 were tested at various doses in SK-BR-3 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated without any transfection reagent with 0.02 μ M, 0.10 μ M, 0.50 μ M, 1.00 μ M, 2.50 μ M, and 10.00 μ M concentrations of antisense oligonucleotide, as specified in Table 60. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, as described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

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The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 60.

Table 60

Dose-dependent antisense inhibition of human STAT3 by free-uptake of ISIS oligonucleotide by SK-BR-3 cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	1 μ M	2.5 μ M	10 μ M	IC ₅₀ (μ M)
455265	22	14	25	19	30	37	>10.0
455269	17	17	21	45	64	67	1.3
455271	0	0	0	11	16	53	9.0
455272	0	0	0	5	12	51	9.6
455291	9	15	31	45	58	76	1.2
455371	16	20	34	37	54	70	1.7
455394	0	2	14	6	30	55	8.3
455429	0	0	0	12	29	57	7.9
455471	0	16	28	24	42	58	2.9
455527	5	15	14	21	35	45	>10.0
455530	0	14	12	14	28	36	>10.0
455536	0	0	0	1	8	26	>10.0
455548	16	14	17	17	20	44	>10.0
455611	19	1	3	21	35	38	>10.0
455703	0	0	0	0	3	33	>10.0
465236	0	7	15	19	37	60	3.8
465237	2	13	22	29	50	67	2.3
465588	5	3	21	18	42	44	>10.0
465740	1	14	0	19	14	39	>10.0
465754	0	0	4	15	39	55	7.7
465830	6	18	23	17	42	67	3.0
466670	21	19	33	35	58	71	1.6
466720	0	0	11	13	27	53	8.7

Example 42: Measurement of viscosity of ISIS antisense oligonucleotides targeting human STAT3

The viscosity of antisense oligonucleotides selected from the studies described in Examples 6-10 was measured with the aim of screening out antisense oligonucleotides which have a viscosity more than 40 cP.

Oligonucleotides having a viscosity greater than 40 cP would be too viscous to be administered to any subject.

ISIS oligonucleotides (32-35 mg) were weighed into a glass vial, 120 μ L of water was added and the antisense oligonucleotide was dissolved into solution by heating the vial at 50°C. Part of (75 μ L) the pre-heated sample was pipetted to a micro-viscometer (Cambridge). The temperature of the micro-viscometer

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was set to 25°C and the viscosity of the sample was measured. Another part (20 µL) of the pre-heated sample was pipetted into 10 mL of water for UV reading at 260 nM at 85°C (Cary UV instrument). The results are presented in Table 61 and indicate that all the antisense oligonucleotides solutions are optimal in their viscosity under the criterion stated above.

5

Table 61
Viscosity of ISIS antisense oligonucleotides targeting human STAT3

ISIS No	Viscosity
455269	6.1
455291	13.6
466371	7.2
455703	17.6
455429	9.3
465237	26.2
465754	19.7
465830	8.1
466670	15.9

Example 43: Effect of ISIS antisense oligonucleotides targeting human STAT3 in cynomolgus monkeys

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Nine antisense oligonucleotides exhibiting a high level of potency were further tested for in cynomolgus monkeys. Antisense oligonucleotide tolerability and pharmacokinetic profile in the liver and kidney was evaluated.

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The study was conducted at the Korea Institute of Toxicology, Republic of Korea. Prior to the study, the monkeys were kept in quarantine for a 30-day time period, during which standard panels of serum chemistry and hematology, examination of fecal samples for ova and parasites, and a tuberculosis test, were conducted to screen out abnormal or ailing monkeys. Nine groups of four randomly assigned male cynomolgus monkeys each were injected subcutaneously thrice per week for the first week, and subsequently twice a week for the next 7 weeks, with 25 mg/kg of ISIS antisense oligonucleotides. A control group of 4 cynomolgus monkeys was injected with PBS subcutaneously thrice per week for the first week, and subsequently twice a week for the next 7 weeks. Terminal sacrifices of all groups were conducted on day 55, which was 48 hours after the last dose.

During the study period, the monkeys were observed daily for signs of illness or distress. Any animal showing adverse effects to the treatment was removed and referred to the veterinarian and Study Director.

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To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, spleen heart, kidney, liver, and gall bladder weights were measured at day 55. Organ weights were measured and treatment group weights were compared to the corresponding PBS control weights

To evaluate the effect of ISIS oligonucleotides on hepatic and kidney function, blood samples were collected from all the study groups. The monkeys were fasted overnight prior to blood collection. Approximately, 1 mL each of blood samples was collected in tubes without any anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Concentrations of transaminases were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Plasma concentrations of ALT (alanine transaminase), AST (aspartate transaminase), and BUN were measured on day 55. C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, was also similarly measured on day 55.

To evaluate the effect of ISIS oligonucleotides on factors involved in inflammation, blood was collected on day 55 from all animals for analyses of complement C3 levels, MIP-1 β cytokine levels, and platelet number.

For complement C3 analysis, approximately 0.5 mL each of blood sample was collected in tubes without anticoagulant for serum separation. For cytokine level analyses, approximately 2 mL each of blood sample was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Complement C3 was measured using an automatic analyzer (Toshiba 200 FR NEO chemistry analyzer, Toshiba co., Japan). Serum was utilized for cytokine analysis using a nine-panel Searchlight Multiplex Array.

For platelet count, approximately 0.5 mL each of blood samples was collected in tubes containing potassium salt of EDTA. Samples were analyzed for platelet count using an ADVIA120 hematology analyzer (Bayer, USA).

The concentration of oligonucleotide was measured in the liver and kidney on day 55. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999), which includes a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTT, designated herein as SEQ ID NO: 2758) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μ g/g.

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Among the 9 antisense oligonucleotides tested, certain antisense oligonucleotides, including ISIS 455269, ISIS 455371, ISIS 455429, and ISIS 455670 met tolerability thresholds for organ weight, ALT, AST, BUN, and hematological parameters.

Example 44: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in MDA-MB-231 cells

ISIS oligonucleotides from the study described in Example 12 were further tested at different doses in MDA-MB-231 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated without any transfection reagent with 0.02 μ M, 0.20 μ M, 1.00 μ M, 5.00 μ M, and 10.00 μ M concentrations of antisense oligonucleotide, as specified in Table 62. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, as described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of STAT3, relative to untreated control cells. The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 62.

Table 62

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by MDA-MB-231 cells

ISIS No	0.02 μ M	0.20 μ M	1.00 μ M	5.00 μ M	10.00 μ M	IC ₅₀ (μ M)
455269	0	3	30	47	59	6.4
455291	1	3	13	41	47	8.3
455371	5	0	10	34	43	>10.0
455429	0	0	22	31	43	>10.0
455703	0	5	13	28	39	>10.0
465237	0	0	22	39	41	>10.0
465754	5	1	22	30	46	>10.0
465830	0	0	17	43	52	7.5
466670	4	7	18	49	56	6.5

Example 45: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in U251-MG cells

ISIS oligonucleotides from the study described in Example 12 were further tested at different doses in U251-MG cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated without any transfection reagent with 0.1 μ M, 1.0 μ M, 5.0 μ M, 10.0 μ M, and 20.0 μ M concentrations of antisense oligonucleotide, as specified in Table 63. After approximately 24 hours, RNA was isolated from the cells

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and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, as described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells. The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 63.

Table 63

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by U251-MG cells

ISIS No	0.1 μ M	1.0 μ M	5.0 μ M	10.0 μ M	20.0 μ M	IC ₅₀ (μ M)
455269	3	16	31	47	56	11.9
455291	0	11	29	42	51	14.1
455371	3	0	25	33	39	>20.0
455429	6	0	25	33	39	>20.0
455703	5	2	13	33	36	>20.0
465237	2	0	7	2	6	>20.0
465754	0	0	8	16	4	>20.0
465830	0	0	18	2	10	>20.0
466670	0	0	18	25	37	>20.0

Example 46: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in A431 cells

ISIS oligonucleotides from the study described in Example 12 were further tested at different doses in A431 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated without any transfection reagent with 0.02 μ M, 0.2 μ M, 1.0 μ M, 5.0 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 64. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, as described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 64. As illustrated in Table 64, the ISIS oligonucleotides were able to penetrate the cell membrane and significantly reduce STAT3 mRNA levels in a dose-dependent manner.

Table 64

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by A431 cells

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ISIS No	0.02 μ M	0.2 μ M	1.0 μ M	5.0 μ M	10.0 μ M	IC ₅₀ (μ M)
455269	41	64	86	86	89	0.15
455291	25	61	83	85	86	0.17
455371	30	65	82	88	92	0.15
455429	15	73	84	87	88	0.19
455703	12	55	72	82	82	0.13
465237	23	72	82	86	87	0.13
465754	0	67	73	79	83	0.15
465830	0	50	67	71	78	0.21
466670	36	79	88	93	94	0.03

Example 47: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in H460 cells

ISIS oligonucleotides from the study described in Example 12 were further tested at different doses in H460 cells. Cells were plated at a density of 4,000 cells per well. Cells were incubated without any transfection reagent with 0.02 μ M, 0.20 μ M, 1.00 μ M, 5.00 μ M, and 10.00 μ M concentrations of antisense oligonucleotide, as specified in Table 65. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS199, as described hereinabove, was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 65. As illustrated in Table 65, the ISIS oligonucleotides were able to penetrate the cell membrane and significantly reduce STAT3 mRNA levels in a dose-dependent manner.

Table 65
Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by H460 cells

ISIS No	0.02 μ M	0.20 μ M	1.00 μ M	5.00 μ M	10.00 μ M	IC ₅₀ (μ M)
455269	3	69	81	92	94	0.1
455291	0	29	79	88	92	0.3
455371	0	20	63	85	89	0.8
455429	3	37	75	87	88	0.6
455703	4	24	69	87	92	0.3
465237	0	20	72	87	89	0.6
465754	10	45	80	91	92	0.2

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465830	10	28	65	82	89	0.7
466670	15	32	71	90	93	0.3

Example 48: Effect of ISIS oligonucleotides targeting STAT3 in the treatment of U251 human glioma cancer xenograft model

BALB/c nude mice inoculated with human U251 glioma tumor cells were treated with ISIS oligonucleotides from the study described in Example 12. The effect of the treatment on tumor growth in the mice was evaluated.

Treatment

BALB/c nude mice were subcutaneously implanted with 1×10^6 tumor cells. On day 4 of the implantation, groups of 4 mice each were administered 50 mg/kg injected intraperitoneally five times a week for 3 and a half weeks of ISIS 455269, ISIS 455291, ISIS 455371, ISIS 455703, ISIS 455429, ISIS 465237, ISIS 465754, ISIS 465830, or ISIS 466670. One group of mice was administered 50 mg/kg injected intraperitoneally five times a week for 3 and a half weeks of the control oligonucleotide, ISIS 141923. One group of mice was administered PBS injected intraperitoneally five times a week for 3 and a half weeks.

Effect on tumor growth

Tumor size was measured twice weekly in two dimensions using a caliper, and tumor volumes were calculated using the formula: $V = 0.5 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The results are presented in Table 66. The data indicates that treatment with ISIS oligonucleotides significantly impeded tumor growth. 'n/a' indicates that the data points for that time point are not available.

Table 66

Effect of antisense inhibition of STAT3 on tumor growth in the U251 xenograft model

	Day 10	Day 14	Day 17	Day 21	Day 23	Day 29	Day 32	Day 35
PBS	205	216	285	381	519	771	937	1,141
ISIS 141923	175	178	296	404	544	719	923	1,027
ISIS 455269	157	151	227	307	349	418	486	542
ISIS 455291	149	169	193	238	297	429	635	610
ISIS 455371	141	169	253	379	375	598	838	912
ISIS 455429	180	160	251	337	427	546	807	897
ISIS 455703	156	161	246	342	414	615	872	991
ISIS 465237	149	166	245	326	350	551	703	744
ISIS 465830	173	205	287	346	383	696	844	825
ISIS 466670	112	172	208	254	274	492	462	669

BIOL0142WO**Example 49: Effect of ISIS 455291 targeting STAT3 in the treatment of an MDA-MB-231 human breast cancer xenograft model**

BALB/c nude mice inoculated with human breast cancer cells MDA-MB-231 were treated with ISIS 455291. The effect of the treatment on tumor growth and tolerability in the mice was evaluated.

Treatment

The study was conducted at Pharmaron Inc (Beijing, P.R. China). The BALB/c nude mice were obtained from Beijing HFK Bio-Technology Co., Ltd. MDA-MB-231 human breast cancer cells were maintained in vitro as a monolayer culture in Leibovitz's L-15 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The cells were maintained at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely sub-cultured twice weekly with trypsin-EDTA treatment. Cells growing an exponential growth phase were harvested and counted for tumor inoculation.

Two groups of eight randomly assigned 6-8 week-old female BALB/c nude mice each were inoculated at the right flank with the MDA-MB-231 tumor fragments (3 mm x 2 mm x 2 mm, which were generated from tumor inoculation passage) for tumor development. Antisense oligonucleotide treatment started at day 11 after tumor inoculation when the mean tumor size reached approximately 100 mm³. One of the groups was injected intraperitoneally twice a week for 3 weeks with 50 mg/kg of ISIS 455291. The other group of mice was injected intraperitoneally twice a week for 3 weeks with PBS, and served as the control group.

All procedures related to animal handling, care, and treatment, were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC). At the time of routine monitoring, the animals were checked for any effects of tumor growth on normal behavior, such as mobility, food consumption, body weight changes and any other abnormal effect.

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis of human STAT3 mRNA levels using primer probe set RTS199, described hereinabove. Murine STAT3 mRNA levels were also measured using primer probe set mSTAT3_LTS00664 (forward sequence CGACAGCTTCCCCATGGA, designated herein as SEQ ID NO: 1513; reverse sequence ATGCCAGTCTTGACTCTCAATC, designated herein as SEQ ID NO: 1514; probe sequence CTGCGGCAGTTCCTGGCACCTT, designated herein as SEQ ID NO: 1515). Results are presented as percent inhibition of STAT3, relative to PBS control, normalized to

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cyclophilin. As shown in Table 67, treatment with ISIS 455291 resulted in reduction of both human and murine STAT3 mRNA in comparison to the PBS control.

Table 67

Inhibition of STAT3 mRNA in the treatment groups relative to the PBS control in the MDA-MB-231 xenograft model

	% inhibition
Human STAT3	91
Murine STAT3	94

Effect on tumor growth

Tumor size was measured twice weekly in two dimensions using a caliper, and tumor volumes were calculated using the formula: $V = 0.536 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The tumor size was utilized for calculations of the T-C and T_V/C_V values. T-C was calculated with T as the median time (in days) required for the tumors in the treatment groups to reach a pre-determined size (900 mm³), and C as the median time (in days) for the tumors in the control group to reach the same size. The T_V/C_V value (expressed as percentage) is an indication of the anti-tumor effectiveness of the ISIS oligonucleotides, where T_V and C_V were the mean volume of the treated and control groups, respectively, on a given day (day 32).

The results are presented in Tables 68 and 69. The data indicates that inhibition of STAT3 mRNA significantly impeded tumor growth.

Table 68

Effect of antisense inhibition of STAT3 on tumor growth in the MDA-MB-231 xenograft model

Days	PBS	ISIS 455291
11	103	103
15	185	156
18	292	205
22	519	320
25	745	437
29	1,332	792
32	1,741	1,075

Table 69

Effect of antisense inhibition of STAT3 on tumor growth inhibition in the MDA-MB-231 xenograft model

Treatment	Tumor Size (mm ³) at day 32	T_V/C_V (%)	T-C at 900 mm ³
PBS	1,741	-	-
ISIS 455291	1,075	62	4

Body weight measurements

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To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body weights were measured on a regular basis during the treatment period. The data is presented in Table 70 and indicate that treatment with ISIS 455291 does not affect the overall body weight of the mice.

Table 70
Body weight measurements of mice in the MDA-MB-231 xenograft model

	Day 11	Day 15	Day 18	Day 22	Day 25	Day 29	Day 32
PBS	22	22	23	23	23	23	24
ISIS 455291	22	22	23	23	24	24	25

Example 50: Effect of ISIS 455291 targeting STAT3 in the treatment of an A431 human epidermoid carcinoma xenograft model

BALB/c nude mice inoculated with human epidermoid cancer cells A431 were treated with ISIS 455291. The effect of the treatment on tumor growth and tolerability in the mice was evaluated.

Treatment

The study was conducted at Pharmaron Inc (Beijing, P.R. China). The BALB/c nude mice were obtained from Beijing HFK Bio-Technology Co., Ltd. A431 human epidermoid carcinoma cells were maintained in vitro as a monolayer culture in DMEM medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The cells were maintained at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely sub-cultured twice weekly with trypsin-EDTA treatment. Cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

Two groups of eight randomly assigned 6-8 week-old female BALB/c nude mice each were inoculated subcutaneously with 5 x 10⁶ A431 tumor cells for tumor development. Antisense oligonucleotide treatment started at day 8 after tumor inoculation when the mean tumor size reached approximately 95 mm³. One of the groups was injected intraperitoneally twice a week for 4 weeks with 50 mg/kg of ISIS 455291. The other group of mice was injected intraperitoneally twice a week for 3 weeks with PBS, and served as the control group.

All procedures related to animal handling, care, and treatment, were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC). At the time of routine monitoring, the animals were checked for any effects of tumor growth on normal behavior, such as mobility, food consumption, body weight changes and any other abnormal effect.

RNA analysis

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RNA was extracted from tumor tissue for real-time PCR analysis of human STAT3 mRNA levels using primer probe set RTS199, described hereinabove. Murine STAT3 mRNA levels were also measured using primer probe set mSTAT3_LTS00664. Results are presented as percent inhibition of STAT3, relative to PBS control, normalized to cyclophilin. As shown in Table 71, treatment with ISIS 455291 resulted in reduction of both human and murine STAT3 mRNA in comparison to the PBS control.

Table 71

Inhibition of STAT3 mRNA in the treatment groups relative to the PBS control in the A431 xenograft model

	% inhibition
Human STAT3	67
Murine STAT3	92

Effect on tumor growth

Tumor size was measured twice weekly in two dimensions using a caliper, and tumor volumes were calculated using the formula: $V = 0.5 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The tumor size was utilized for calculations of the T-C and T_V/C_V values. T-C was calculated with T as the median time (in days) required for the tumors in the treatment groups to reach a pre-determined size (800 mm³), and C as the median time (in days) for the tumors in the control group to reach the same size. The T_V/C_V value (expressed as percentage) is an indication of the anti-tumor effectiveness of the ISIS oligonucleotides, where T_V and C_V were the mean volume of the treated and control groups, respectively, on a given day (day 33).

The results are presented in Tables 72 and 73. The data indicates that inhibition of STAT3 mRNA impeded tumor growth.

Table 72

Effect of antisense inhibition of STAT3 on tumor growth in the A431 xenograft model

Days	PBS	ISIS 455291
8	94	95
14	178	173
17	308	242
21	528	393
24	682	572
28	875	759
31	1,071	984
33	1,210	1,112

Table 73

Effect of antisense inhibition of STAT3 on tumor growth inhibition in the A431 xenograft model

Treatment	Tumor Size (mm ³) at day 33	T_V/C_V (%)	T-C at 800 mm ³
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PBS	1,210	-	-
ISIS 455291	1,112	92	2

Body weight measurements

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body weights were measured on a regular basis during the treatment period. The data is presented in Table 74 and indicate that treatment with ISIS 455291 does not affect the overall body weight of the mice.

Table 74
Body weight measurements of mice in the A431 xenograft model

	Day 8	Day 14	Day 17	Day 21	Day 24	Day 28	Day 31	Day 33
PBS	20	20	20	21	21	21	22	22
ISIS 455291	20	21	21	22	22	22	23	23

Example 51: Effect of ISIS 455291 targeting STAT3 in the treatment of an NCI-H460 human non-small cell lung cancer (NSCLC) xenograft model

BALB/c nude mice inoculated with human NCI-H460 human NSCLC were treated with ISIS 455291. The effect of the treatment on tumor growth and tolerability in the mice was evaluated.

Treatment

The study was conducted at Pharmaron Inc (Beijing, P.R. China). The BALB/c nude mice were obtained from Beijing HFK Bio-Technology Co., Ltd. NCI-H460 human NSCLC cells were maintained in vitro as a monolayer culture in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The cells were maintained at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely sub-cultured twice weekly with trypsin-EDTA treatment. Cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

Two groups of eight randomly assigned 6-8 week-old female BALB/c nude mice each were inoculated subcutaneously with 2×10^6 NCI-H460 tumor cells for tumor development. Antisense oligonucleotide treatment started at day 6 after tumor inoculation when the mean tumor size reached approximately 100 mm³. One of the groups was injected intraperitoneally twice a week for 3 weeks with 50 mg/kg of ISIS 455291. The other group of mice was injected intraperitoneally twice a week for 3 weeks with PBS, and served as the control group.

All procedures related to animal handling, care, and treatment, were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC). At the time of routine

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monitoring, the animals were checked for any effects of tumor growth on normal behavior, such as mobility, food consumption, body weight changes and any other abnormal effect.

Effect on tumor growth

Tumor size was measured twice weekly in two dimensions using a caliper, and tumor volumes were calculated using the formula: $V = 0.5 \times a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. The tumor size was utilized for calculations of the T-C and T_V/C_V values. T-C was calculated with T as the median time (in days) required for the tumors in the treatment groups to reach a pre-determined size (1,500 mm³), and C as the median time (in days) for the tumors in the control group to reach the same size. The T_V/C_V value (expressed as percentage) is an indication of the anti-tumor effectiveness of the ISIS oligonucleotides, where T_V and C_V were the mean volume of the treated and control groups, respectively, on a given day (day 20).

The results are presented in Tables 75 and 76. The data indicates that inhibition of STAT3 significantly impeded tumor growth.

Table 75

Effect of antisense inhibition of STAT3 on tumor growth in the NCI-H460 xenograft model

Days	PBS	ISIS 455291
6	104	104
8	303	180
11	746	408
13	1,175	620
15	1,642	819
18	2,277	1,320
20	2,859	1,812
22	-	2,330

Table 76

Effect of antisense inhibition of STAT3 on tumor growth inhibition in the NCI-H460 xenograft model

Treatment	Tumor Size (mm ³) at day 20	T_V/C_V (%)	T-C at 800 mm ³
PBS	1,210	-	-
ISIS 455291	1,812	63	4

20 *Body weight measurements*

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body weights were measured on a regular basis during the treatment period. The data is presented in Table 77 and indicate that treatment with ISIS 455291 does not affect the overall body weight of the mice.

Table 77

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Body weight measurements of mice in the NCI-H460 xenograft model

	Day 6	Day 8	Day 11	Day 13	Day 15	Day 18	Day 20	Day 22
PBS	20	20	20	20	20	20	21	-
ISIS 455291	20	20	20	20	20	19	20	20

5 **Example 52: Effect of antisense inhibition of human STAT3 in a human glioblastoma orthotopic mouse model**

NU/J mice orthotopically implanted with human glioblastoma cells were treated with ISIS 455291, a 5-10-5 MOE gapmer having a sequence of CAGCAGATCAAGTCCAGGGA (SEQ ID NO: 1590). The effect of the treatment on tumor growth and tolerability in the mice was evaluated.

10 *Treatment*

Thirty NU/J mice were stereotactically implanted in the right frontal lobe with 5×10^5 U-87 MG-luc2 cells. On day 15 after tumor cell implantation, 15 of these mice were dosed intracranially with a bolus injection at the site of tumor implantation with 100 μ g of ISIS 455291, which was dissolved in 2 μ L of PBS. The remaining 15 mice were dosed intracranially with a bolus injection at the site of tumor implantation with 2 μ L of PBS. The second group of mice served as the control group.

Analysis

On day 18 after tumor transplantation, five mice from each group were euthanized by CO₂ inhalation and brain samples were collected for RNA analysis. RNA was extracted from tumor tissue for real-time PCR analysis of human STAT3 mRNA levels using primer probe set RTS199, described hereinabove. Treatment with ISIS 455291 resulted in 27% reduction of human STAT3 mRNA in the tumor tissue in comparison to the PBS control.

The remaining mice in each group were monitored regularly up to 2 weeks for survival analysis. The median survival for the PBS control group was 30.5 days. The medial survival for the ISIS oligonucleotide-treated mice was 35 days. The P value was 0.2088.

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Example 53: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in PC9 cells

ISIS 455703 and ISIS 455291, from the studies described above, were further tested at different doses in PC9 cells, a non small cell lung carcinoma cell line. Cells were plated at a density of 3,000 cells per well. Cells were incubated with 0.02 μ M, 0.1 μ M, 0.5 μ M, 2.5 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 78. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCCGCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 78. As illustrated in Table 78, ISIS 455703 and ISIS 455291 were able to penetrate the cell membrane.

Table 78

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by PC9 cells

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	2.5 μ M	10.0 μ M	IC_{50} (μ M)
455703	6	5	17	50	49	9.0
455291	0	0	42	67	75	1.2

Example 54: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in C42B cells

ISIS 455291, from the studies described above, was further tested at different doses in C42B cells, a prostate cancer cell line. Cells were plated at a density of 3,000 cells per well. Cells were incubated with 0.02 μ M, 0.1 μ M, 0.5 μ M, 2.5 μ M, and 10.0 μ M concentrations of antisense oligonucleotide, as specified in Table 79. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence GAGGCCCCGCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence

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TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated
 5 herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

As illustrated in Table 79, ISIS 455291 was able to penetrate the cell membrane.

Table 79

10 Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by C42B cells

ISIS No	0.02 μM	0.1 μM	0.5 μM	2.5 μM	10.0 μM
455291	0	0	17	10	41

Example 55: Dose-dependent antisense inhibition of STAT3 following free uptake of antisense oligonucleotide in Colo201 cells

15 ISIS 455291, from the studies described above, was further tested at different doses in Colo201 cells, a colorectal cancer cell line. Cells were plated at a density of 3,000 cells per well. Cells were incubated with 0.02μM, 0.1 μM, 0.5 μM, 2.5 μM, and 10.0 μM concentrations of antisense oligonucleotide, as specified in Table 80. After approximately 24 hours, RNA was isolated from the cells and STAT3 mRNA levels were measured by quantitative real-time PCR. Human STAT3 primer probe set RTS2033 (forward sequence
 20 GAGGCCCGCCCAACA, designated herein as SEQ ID NO: 1520; reverse sequence TTCTGCTAATGACGTTATCCAGTTTT, designated herein as SEQ ID NO: 1521; probe sequence CTGCCTAGATCGGC, designated herein as SEQ ID NO: 1522) was used to measure mRNA levels. STAT3 mRNA levels were adjusted according to content of beta-actin, a housekeeping gene, as measured by human primer probe set HTS5002 (forward sequence CGGACTATGACTTAGTTGCGTTACA, designated
 25 herein as SEQ ID NO: 1529; reverse sequence GCCATGCCAATCTCATCTTGT, designated herein as SEQ ID NO: 1530; probe sequence CCTTTCTTGACAAAACCTAACTTGCGCAGA, designated herein as SEQ ID NO: 1531). Results are presented as percent inhibition of STAT3, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 80. As illustrated in Table 29, ISIS 455291 was able to penetrate the cell membrane.

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Table 80

Dose-dependent antisense inhibition of STAT3 mRNA levels by free-uptake of ISIS oligonucleotide by Colo201 cells

ISIS No	0.02 μM	0.1 μM	0.5 μM	2.5 μM	10.0 μM	IC ₅₀ (μM)
455291	21	18	34	52	81	1.2

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What is claimed is:

1. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 3008 to 3033 of SEQ ID NO: 1, wherein the nucleobase sequence is complementary to SEQ ID NO: 1.
2. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 3016 to 3031 of SEQ ID NO: 1, wherein the nucleobase sequence is complementary to SEQ ID NO: 1.
3. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 6476 to 6491 of SEQ ID NO: 2, wherein the nucleobase sequence is complementary to SEQ ID NO: 2.
4. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of nucleobases 250-286; 250-285; 264-285; 264-282; 728-745; 729-745; 729-744; 787-803; 867-883; 955-978; 1146-1170; 1896-1920; 1899-1920; 1899-1919; 1899-1918; 1899-1916; 1901-1916; 1946-1963; 1947-1963; 2155-2205; 2155-2187; 2156-2179; 2204-2221; 2681-2696; 2699-2716; 3001-3033; 3008-3033, 3010-3033, 3010-3032, 3015-3033, 3015-3032, 3015-3031, 3016-3033, 3016-3032, 3016-3033; 3452-3499; 3460-3476; 3583-3608; 3591-3616; 3595-3615; 3595-3614; 3595-3612; 3675-3706; 3713-3790; 3715-3735; 3833-3878; 3889-3932; 3977-4012; 4067-4100; 4225-4256; 4234-4252; 4235-4252; 4235-4251; 4236-4252; 4306-4341; 4431-4456; 4439-4454; 4471-4510; 4488-4505; 4530-4558; 4539-4572; 4541-4558; 4636-4801; 4782-4796; 4800-4823; 4811-4847; 4813-4859; 4813-4815; 4813-4831; 4827-4859; 4827-4844; or 4842-4859 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 1.
5. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary

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to an equal length portion of nucleobases 2668-2688; 2703-2720; 5000-5021; 5001-5017; 5697-5722; 5699-5716; 6475 - 6490; 6475 - 6491; 6476 - 6491; 7682-7705; 8078-8097; 8079-8095; 9862-9811; 9870-9897; 9875-9893; 9875-9891; 9877-9893; 11699-11719; 12342-12366; 12345-12364; 12346-12364; 12347-12364; 12353-12380; 12357-12376; 12358-12376; 12358-12373; 12360-12376; 14128-14148; 16863-16883; 46091-46111; 50692-50709; 50693-50709; 50693-50708; 61325-61349; 66133-66157; 66136-66157; 66136 - 66155; 66136-66153; 66138-66153; 66184-66200; 67067-67083; 4171-74220; 74199-74220; 74202-74220; 74171-74219; 74199-74219; 74202-74219; 74171-74218; 74199-74218; 74202-74218; 74723-74768; 74764-74803; 74782-74802; 74782-74801; 74782-74800; 74782-74799; 74783-74802; 74783-74801; 74783-74800; 74783-74799; 74862-74893; 74900-74977; 74902-74922; 74902-74920; 75070-75119; 75164-75199; 75254-75287; 75412-75443; 75421-75439; 75422-75439; 75422-75438; 75423-75439; 75423-75438; 75493-75528; 75616-75643; 75626-75641; 75658-75699; 75676-75692; 75717-75745; 75726-75759; 75726-75745; 75727-75745; 75728-75745; 75831-75988; 75852-75969; 75969-75984; 75987-76056; 76000-76046; 76000-76032; 76000-76018; 76014-76046; 76014-76032; 76029-76046; or 76031-76046 of SEQ ID NO: 2, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 2.

6. The compound of claim 1 or 2, wherein the nucleobase sequence of the modified oligonucleotide comprises the sequence of SEQ ID NO: 245.
7. The compound of claim 1 or 2, wherein the nucleobase sequence of the modified oligonucleotide consists of the sequence of SEQ ID NO: 245.
8. The compound of claim 3, wherein the nucleobase sequence of the modified oligonucleotide comprises the sequence of SEQ ID NO: 413.
9. The compound of claim 3, wherein the nucleobase sequence of the modified oligonucleotide consists of the sequence of SEQ ID NO: 413.
10. The compound of any of claims 1-9, wherein the modified oligonucleotide is 100% complementary to SEQ ID NO: 1 or 2.
11. The compound of any of claims 1-10, consisting of a single-stranded modified oligonucleotide.

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12. The compound of any of claims 1-11, wherein at least one internucleoside linkage is a modified internucleoside linkage.
13. The compound of claim 12, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.
14. The compound of any of claims 1-13, wherein at least one nucleoside comprises a modified sugar.
15. The compound of claim 14, wherein at least one modified sugar is a bicyclic sugar.
16. The compound of claim 15, wherein the bicyclic sugar comprises a 4'-CH₂-O-2' bridge.
17. The compound of claim 15, wherein the bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.
18. The compound of claim 14, wherein the modified sugar comprises a 2'-O(CH₂)₂-OCH₃ group.
19. The compound of claim 14, wherein the modified sugar comprises a 2'-O-CH₃ group.
20. The compound of any of claims 1-19, wherein at least one nucleoside comprises a modified nucleobase.
21. The compound of claim 20, wherein the modified nucleobase is a 5'-methylcytosine.
22. The compound of any of claims 1-13, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 1 to 5 linked nucleosides;
a 3'-wing consisting of 1 to 5 linked nucleosides;
a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and
wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or one 2'-substituted nucleoside.
23. The compound of any of claims 1-13, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 1 to 5 linked nucleosides;
a 3'-wing consisting of 1 to 5 linked nucleosides;

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a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside and at least one 2'-substituted nucleoside.

24. The compound of claim 22 or 23, wherein the 2'-substituted nucleoside comprises any of the group consisting of a 2'-O(CH₂)₂-OCH₃ group or a 2'-O-CH₃ group.

25. The compound of claim 22 or 23, wherein the bicyclic nucleoside comprises any of the group consisting of a 4'-CH₂-O-2' bridge and a 4'-CH(CH₃)-O-2' bridge.

26. The compound of any of claims 1-13, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 3 linked nucleosides;
a 3'-wing consisting of 3 linked nucleosides;
a gap between the 5'-wing and the 3'-wing consisting of 10 linked 2'-deoxynucleosides;
wherein each nucleoside of each of the 5'-wing and the 3'-wing comprises a constrained ethyl nucleoside;
wherein each internucleoside linkage is a phosphorothioate linkage; and
wherein each cytosine is a 5'-methylcytosine.

27. A compound, comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 245.

28. A compound, comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of the nucleobase sequence of SEQ ID NO: 413.

29. A compound, comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 9-426, 430-442, 445-464, 471-498, 500-1034, 1036-1512, and 1541-2757.

30. The compound of any of claims 26-29, consisting of a single-stranded modified oligonucleotide.

31. The compound of any of claims 27-30, wherein at least one internucleoside linkage is a modified internucleoside linkage.

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32. The compound of claim 31, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.
33. The compound of any of claims 27-32, wherein at least one nucleoside comprises a modified sugar.
34. The compound of claim 33, wherein at least one modified sugar is a bicyclic sugar.
35. The compound of claim 34, wherein the bicyclic sugar comprises a 4'-CH₂-O-2' bridge.
36. The compound of claim 35, wherein the bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.
37. The compound of claim 33, wherein the modified sugar comprises a 2'-O(CH₂)₂-OCH₃ group.
38. The compound of claim 33, wherein the modified sugar comprises a 2'-O-CH₃ group.
39. The compound of any of claims 27-38, wherein at least one nucleoside comprises a modified nucleobase.
40. The compound of claim 38, wherein the modified nucleobase is a 5'-methylcytosine.
41. The compound of any of claims 27-30, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 1 to 5 linked nucleosides;
a 3'-wing consisting of 1 to 5 linked nucleosides;
a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and
wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or 2'-substituted nucleoside.
42. The compound of any of claims 27-30, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 1 to 5 linked nucleosides;
a 3'-wing consisting of 1 to 5 linked nucleosides;
a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides; and

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wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside and at least one 2'-substituted nucleoside.

43. The compound of claim 41 or 42, wherein the 2'-substituted nucleoside comprises any of the group consisting of a 2'-O(CH₂)₂-OCH₃ group or a 2'-O-CH₃ group.

44. The compound of claim 41 or 42, wherein the bicyclic nucleoside comprises any of the group consisting of a 4'-CH₂-O-2' bridge and a 4'-CH(CH₃)-O-2' bridge.

45. The compound of any of claims 27-30, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 3 linked nucleosides;
a 3'-wing consisting of 3 linked nucleosides;
a gap between the 5'-wing and the 3'-wing consisting of 10 linked 2'-deoxynucleosides;
wherein each nucleoside of each of the 5'-wing and the 3'-wing comprises a constrained ethyl nucleoside;
wherein each internucleoside linkage is a phosphorothioate linkage; and
wherein each cytosine is a 5'-methylcytosine.

46. A compound comprising:
a modified oligonucleotide consisting of 12 to 22 linked nucleosides, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 1 to 5 linked nucleosides;
a 3'-wing consisting of 1 to 5 linked nucleosides;
a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides;
wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or a 2'-substituted nucleoside;
wherein the nucleobase sequence of the modified oligonucleotide is complementary to an equal length portion of nucleobases 3016 to 3031 of the nucleobase sequence of SEQ ID NO: 1; and
wherein the compound inhibits expression of STAT3 mRNA expression.

47. A compound comprising:
a modified oligonucleotide consisting of 12 to 22 linked nucleosides, wherein the modified oligonucleotide comprises:
a 5'-wing consisting of 1 to 5 linked nucleosides;
a 3'-wing consisting of 1 to 5 linked nucleosides;

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a gap between the 5'-wing and the 3'-wing consisting of 8 to 12 linked 2'-deoxynucleosides;
wherein at least one of the 5'-wing and the 3'-wing comprises at least one bicyclic nucleoside or a 2'-substituted nucleoside;

wherein the nucleobase sequence of the modified oligonucleotide is complementary to an equal length portion of nucleobases 6476 to 6491 of the nucleobase sequence of SEQ ID NO: 2; and
wherein the compound inhibits expression of STAT3 mRNA expression.

48. The compound of claim 46 or 47, wherein at least one of the 5'-wing and the 3'-wing comprises at least one 2'-deoxynucleoside.

49. The compound of any of claims 46-48, consisting of a single-stranded modified oligonucleotide.

50. The compound of any of claim 46-49, comprising at least one bicyclic nucleoside.

51. The compound of claim 50, wherein at least one bicyclic nucleoside comprises a 4'-CH(CH₃)-O-2' bridge.

52. The compound of claim 51, wherein each bicyclic nucleoside comprises a 4'-CH(CH₃)-O-2' bridge.

53. The compound of claim 50, wherein at least one bicyclic nucleoside comprises a 4'-CH₂-O-2' bridge.

54. The compound of claim 53, wherein each bicyclic nucleoside comprises a 4'-CH₂-O-2' bridge.

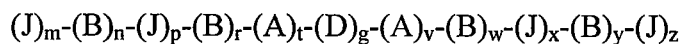
55. The compound of any of claim 46-54, comprising at least one 2'-substituted nucleoside.

56. The compound of claim 55, wherein at least one 2'-substituted nucleoside comprises a 2'-O(CH₂)₂-OCH₃ group.

57. The compound of claim 56, wherein each 2'-substituted nucleoside comprises a 2'-O(CH₂)₂-OCH₃ group.

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58. The compound of claim 55, wherein at least one 2'-substituted nucleoside comprises a 2'-O-CH₃ group.
59. The compound of claim 58, wherein each 2'-substituted nucleoside comprises a 2'-O-CH₃ group.
60. The compound of any of claims 46-59, wherein at least one internucleoside linkage is a modified internucleoside linkage.
61. The compound of claim 60, wherein each modified internucleoside linkage is a phosphorothioate linkage.
62. The compound of any of claims 46-61, wherein at least one nucleoside comprises a modified nucleobase.
63. The compound of claim 62, wherein the modified nucleobase is a 5'-methylcytosine.
64. The compound of any of claims 46-49, wherein the modified oligonucleotide has a sugar motif described by Formula A as follows:



wherein:

each A is independently a 2'-substituted nucleoside;

each B is independently a bicyclic nucleoside;

each J is independently either a 2'-substituted nucleoside or a 2'-deoxynucleoside;

each D is a 2'-deoxynucleoside;

m is 0-4; n is 0-2; p is 0-2; r is 0-2; t is 0-2; v is 0-2; w is 0-4; x is 0-2; y is 0-2; z is 0-4; g is 6-14;

provided that:

at least one of m, n, and r is other than 0;

at least one of w and y is other than 0;

the sum of m, n, p, r, and t is from 2 to 5; and

the sum of v, w, x, y, and z is from 2 to 5.

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65. The compound of any of claims 46-49, wherein the modified oligonucleotide has a sugar motif of any of the group consisting of:

k-d(10)-k
 e-d(10)-k
 k-d(10)-e
 k-k-d(10)-k-k
 k-k-d(10)-e-e
 e-e-d(10)-k-k
 k-k-k-d(10)-k-k-k
 e-e-e-d(10)-k-k-k
 k-k-k-d(10)-e-e-e
 k-k-k-d(10)-k-k-k
 e-k-k-d(10)-k-k-e
 e-e-k-d(10)-k-k-e
 e-d-k-d(10)-k-k-e
 e-k-d(10)-k-e-k-e
 k-d(10)-k-e-k-e-e
 e-e-k-d(10)-k-e-k-e
 e-d-d-k-d(9)-k-k-e
 e-e-e-e-d(9)-k-k-e

wherein, k is a constrained ethyl nucleoside, e is a 2'-MOE substituted nucleoside, and d is a 2'-deoxynucleoside.

66. A method of treating a hyperproliferative disease in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 9-426, 430-442, 445-464, 471-498, 500-1034, 1036-1512, , and 1541-2757.

67. A method of treating a hyperproliferative disease in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 245.

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68. A method of treating a hyperproliferative disease in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 413.
69. The method of any of claims 66-68, wherein the administering reduces tumor size in the animal.
70. The method of any of claims 66-68, wherein the administering reduces tumor volume in the animal.
71. The method of any of claims 66-68, wherein the administering prevents metastasis in the animal.
72. The method of any of claims 66-68, wherein the administering prolongs survival of the animal.
73. The method of any one of claims 66-68, wherein the administering reduces cachaxia in the animal.
74. A method of reducing expression of STAT3 in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 9-426, 430-442, 445-464, 471-498, 500-1034, 1036-1512, .
75. A method of reducing expression of STAT3 in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 245.
76. A method of reducing expression of STAT3 in an animal, comprising administering to an animal in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of SEQ ID NO: 413.